# RAS: What Can be Learned from Clinical Studies?

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Developmental Therapeutics Branch
Center for Cancer Research NCI

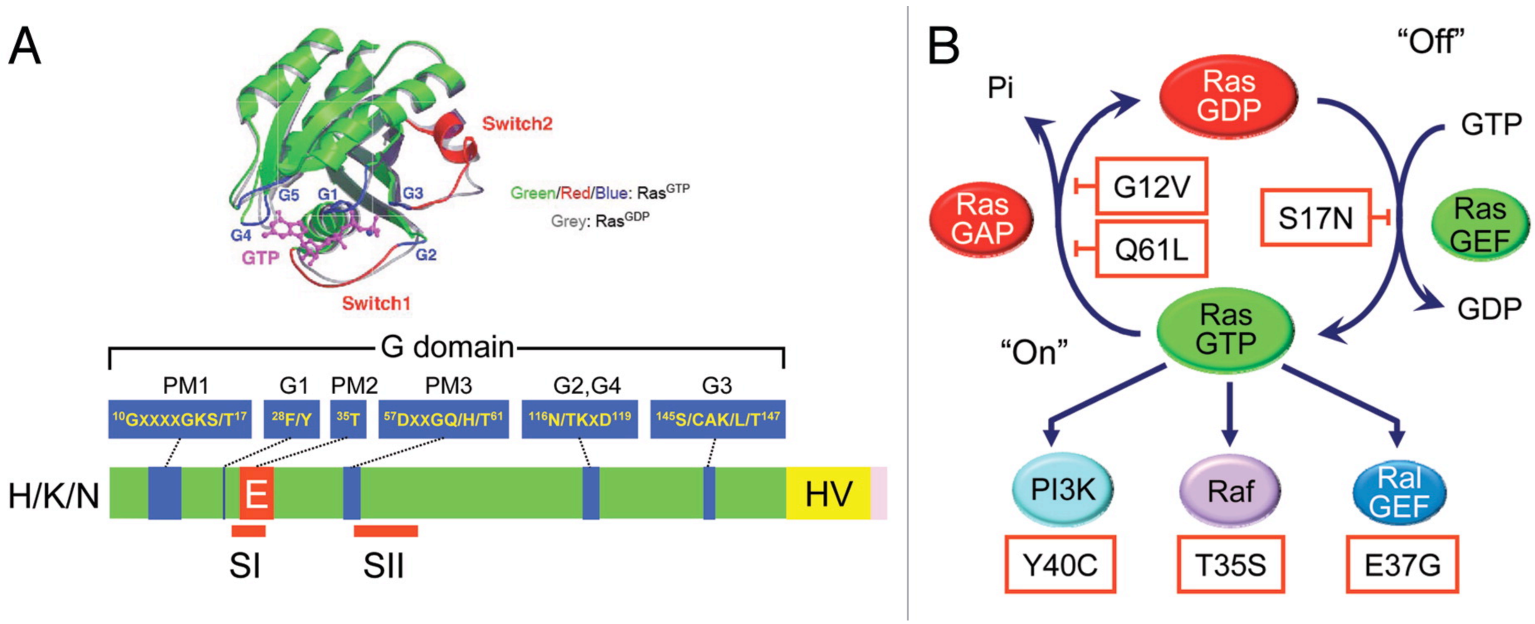
# RAS: is it the Holy Grail?

- How often do we find it?
- In what diseases – and what does this tell us?
- What is its role in outcome?
- What is its role in affecting other therapies?
- What is its role in the origin of cancer?
- What approaches have been attempted?
- How much benefit can we expect from targeting RAS?

# Timeline to Medical Oncology

- 1964 – Harvey sarcoma virus
- 1967 – Kirsten sarcoma virus
- 1976 – Viral oncogene transduced from a normal cellular counterpart
- 1979 – p21 protein
- 1979 – RAS is a GDP and GTP binding protein
- 1982 – Viral H-*ras and* K-*ras* genes have a normal human cellular counterpart
- 1982 – Overexpressed human H-Ras transforms NIH3T3 cells
- 1982 – Bladder cancer *HRAS* gene is activated by a codon 12 mutation
- 1983 – *KRAS*, *NRAS* activating mutations
- 1983 – Ras transformation of primary cells requires cooperating genes
- 1988 – Ras crystal structure
- 1989 – Ras farnesylation described
- 1992 – MEK signaling
- 1993 – Farnesyltransferase inhibitors block growth of H-Ras transformed cells

# RAS: a Small GTPase



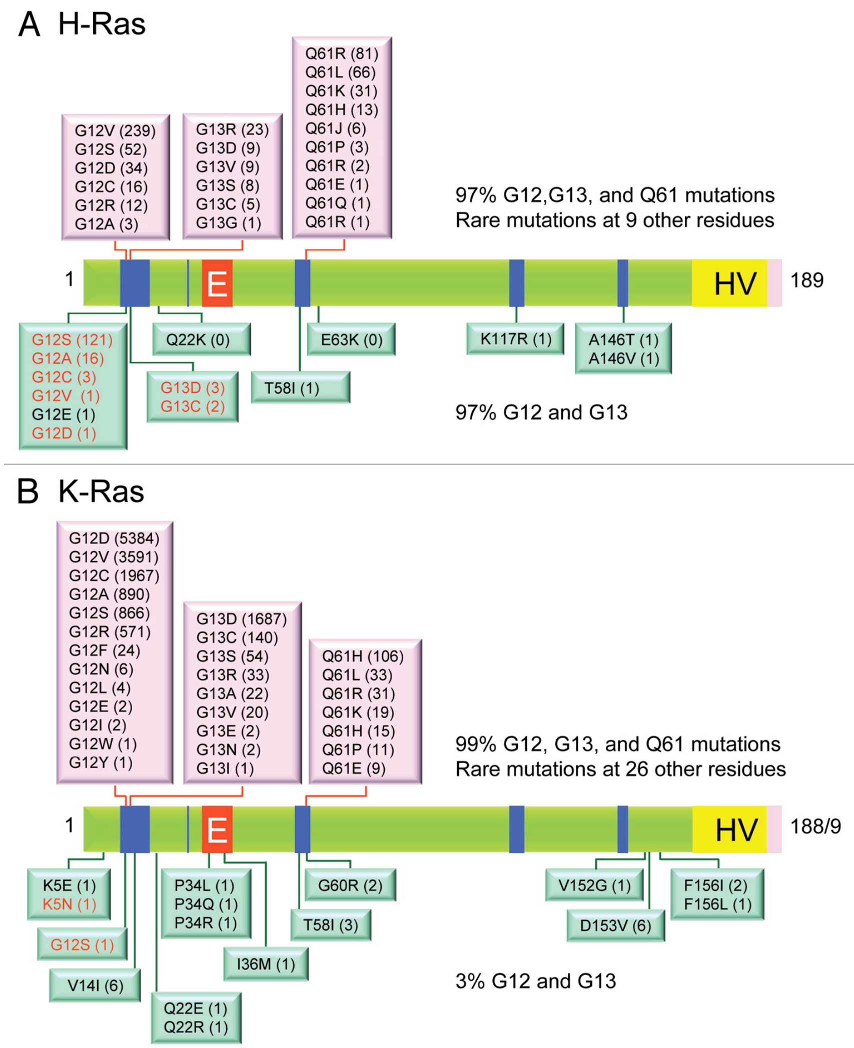

Cox AD and Der CJ. Ras history: The saga continues. Small GTPases 1:1, 2010

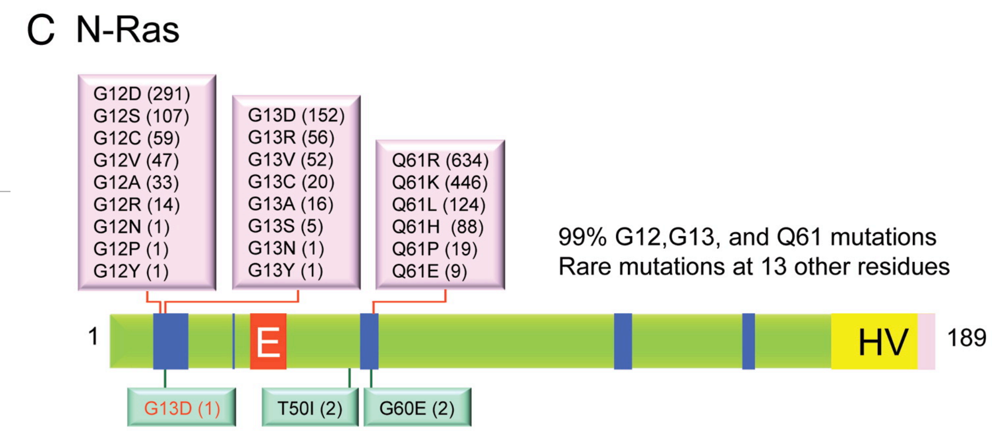
# Incidence of RAS Mutations in Cancer

| | HRAS | | | KRAS | | | NRAS | | | Pan-Ras |
|---|---|---|---|---|---|---|---|---|---|---|
| **Primary tissue** | + | *n* | % | + | *n* | % | + | *n* | % | % |
| Adrenal gland | 1 | 135 | <1% | 1 | 210 | <1% | 7 | 170 | 4% | 5% |
| Autonomic ganglia | 0 | 63 | 0% | 2 | 63 | 3% | 7 | 102 | 7% | 10% |
| Biliary tract | 0 | 151 | 0% | 460 | 1,471 | 31% | 3 | 213 | 1% | 33% |
| Bone | 3 | 147 | 2% | 2 | 165 | 1% | 0 | 143 | 0% | 3% |
| Breast | 5 | 542 | <1% | 20 | 544 | 4% | 7 | 330 | 2% | 7% |
| Central nervous system | 0 | 942 | 0% | 8 | 1,032 | <1% | 8 | 995 | <1% | 2% |
| Cervix | 23 | 264 | 9% | 46 | 637 | 7% | 2 | 132 | 2% | 17% |
| Endometrium | 3 | 291 | 1% | 298 | 2,108 | 14% | 1 | 279 | <1% | 16% |
| Hematopoietic/lymphoid | 8 | 3,074 | <1% | 277 | 5,757 | 5% | 877 | 8,540 | 10% | 15% |
| Kidney | 1 | 273 | <1% | 4 | 617 | <1% | 2 | 435 | <1% | 1% |
| Large intestine | 2 | 617 | <1% | 9,671 | 29,183 | 33% | 26 | 1,056 | 3% | 36% |
| Liver | 0 | 270 | 0% | 21 | 450 | 5% | 8 | 310 | 3% | 7% |
| Lung | 9 | 1,957 | <1% | 2,533 | 14,632 | 17% | 26 | 2,678 | 1% | 19% |
| Esophagus | 2 | 161 | 1% | 13 | 359 | 4% | 0 | 161 | 0% | 5% |
| Ovary | 0 | 94 | 0% | 406 | 2,934 | 14% | 5 | 111 | 5% | 18% |
| Pancreas | 0 | 221 | 0% | 3,127 | 5,169 | 61% | 5 | 248 | 2% | 63% |
| Prostate | 29 | 500 | 6% | 82 | 1,024 | 8% | 8 | 530 | 2% | 15% |
| Salivary gland | 24 | 161 | 15% | 5 | 170 | 3% | 0 | 45 | 0% | 18% |
| Skin | 120 | 1,940 | 6% | 38 | 1,405 | 3% | 858 | 4,742 | 18% | 27% |
| Small intestine | 0 | 5 | 0% | 62 | 316 | 20% | 0 | 5 | 0% | 20% |
| Stomach | 14 | 384 | 4% | 163 | 2,571 | 6% | 5 | 215 | 2% | 12% |
| Testis | 5 | 130 | 4% | 17 | 432 | 4% | 8 | 283 | 3% | 11% |
| Thymus | 1 | 46 | 2% | 4 | 186 | 2% | 0 | 46 | 0% | 4% |
| Thyroid | 117 | 3,601 | 3% | 137 | 4,628 | 3% | 312 | 4,126 | 8% | 14% |
| Upper aerodigestive tract | 101 | 1,083 | 9% | 52 | 1,535 | 3% | 24 | 807 | 3% | 16% |
| Urinary tract | 138 | 1,242 | 11% | 29 | 591 | 5% | 9 | 398 | 2% | 18% |
| **Total** | 606 | 18,294 | 3% | 17,478 | 78,189 | 22% | 2,208 | 27,100 | 8% | 16% |

**Prior et al. Cancer Res (2012) 72:2457-67**

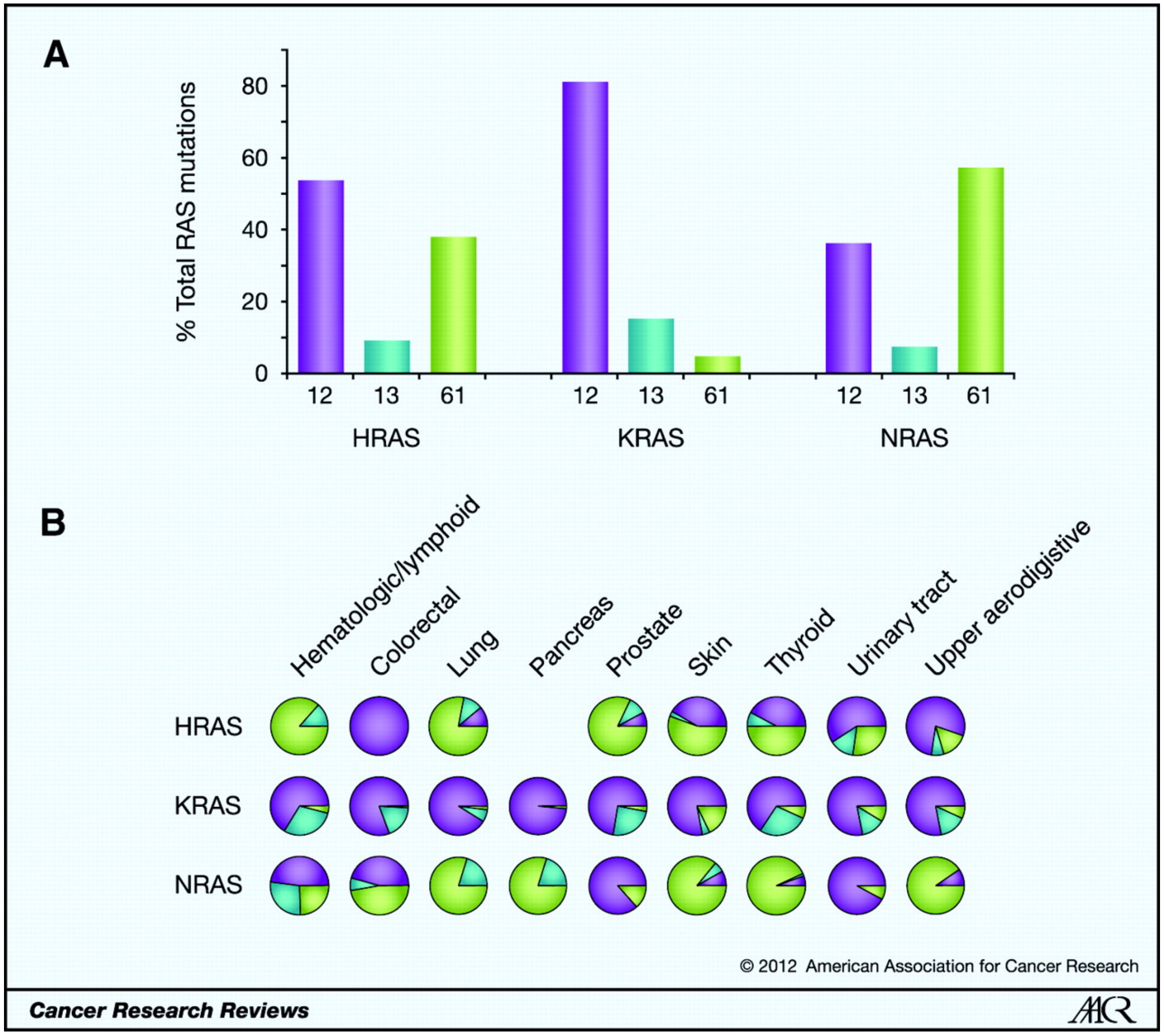
# Most Cancer Mutations Occur in 3 Residues



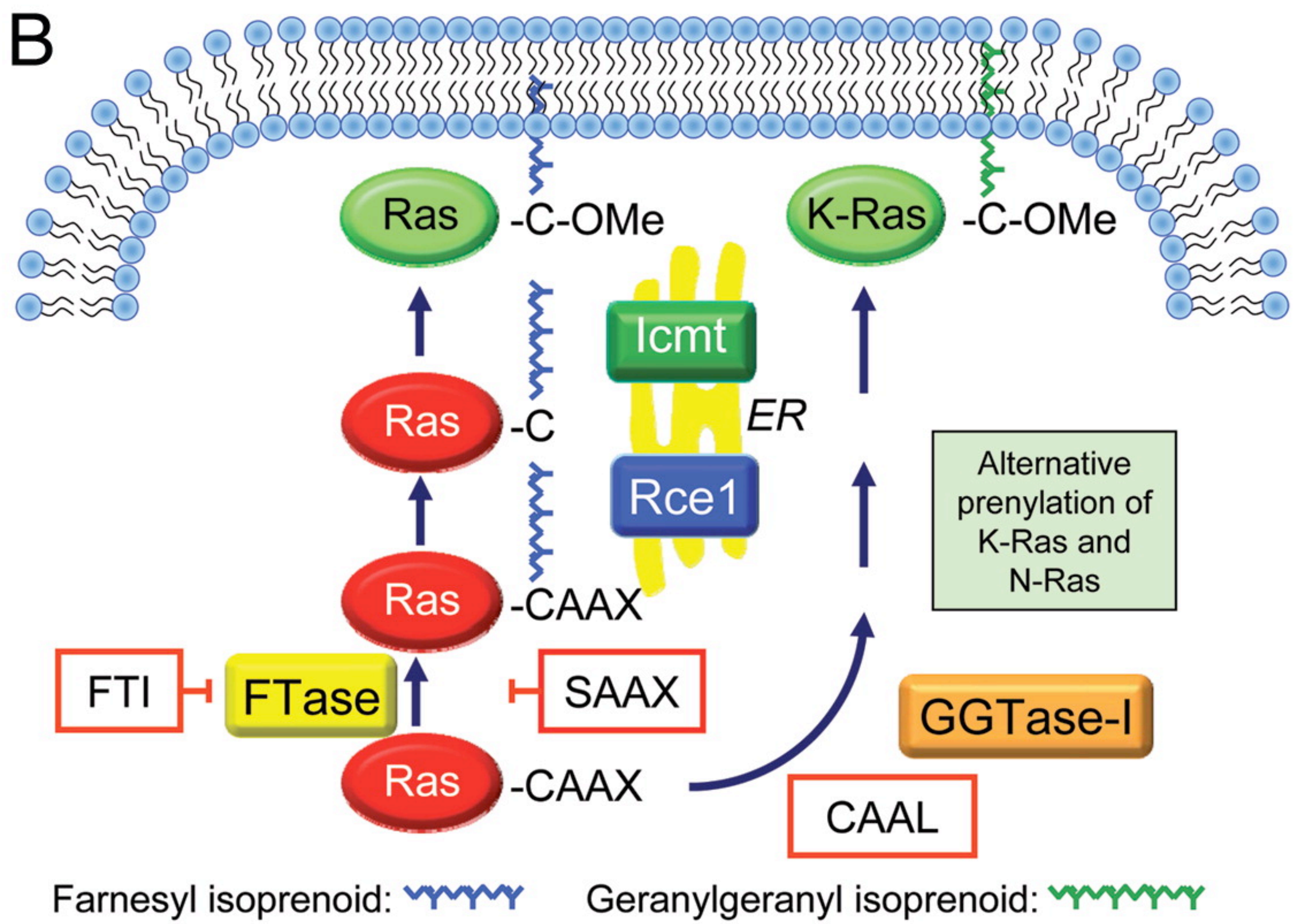

Cox AD and Der CJ. Ras history: The saga continues. Small GTPases 1:1, 2010

# Ras-isoform–specific codon mutation bias

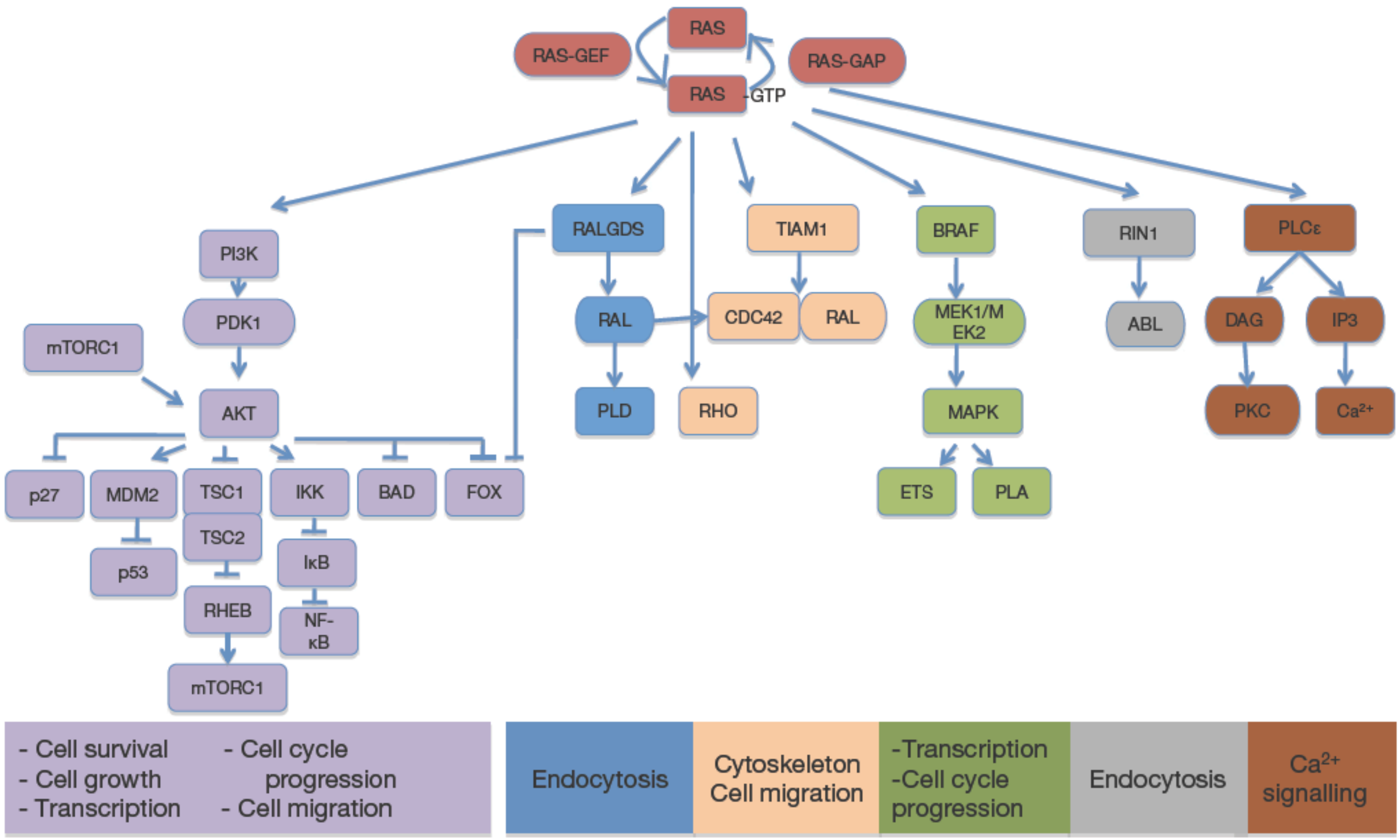



Prior I A et al. Cancer Res 2012;72:2457-2467

# Farnesylation and geranylgeranylation are post-translational modifications required to recruit RAS to the cell membrane



# RAS mediates signaling through at least six different intracellular pathways




**de Castro Carpeño J, et al. Transl Lung Cancer Res 2013;2(2):142-151**

# Incidence of RAS Mutations in Cancer

| | HRAS | | | KRAS | | | NRAS | | | Pan-Ras |
|---|---|---|---|---|---|---|---|---|---|---|
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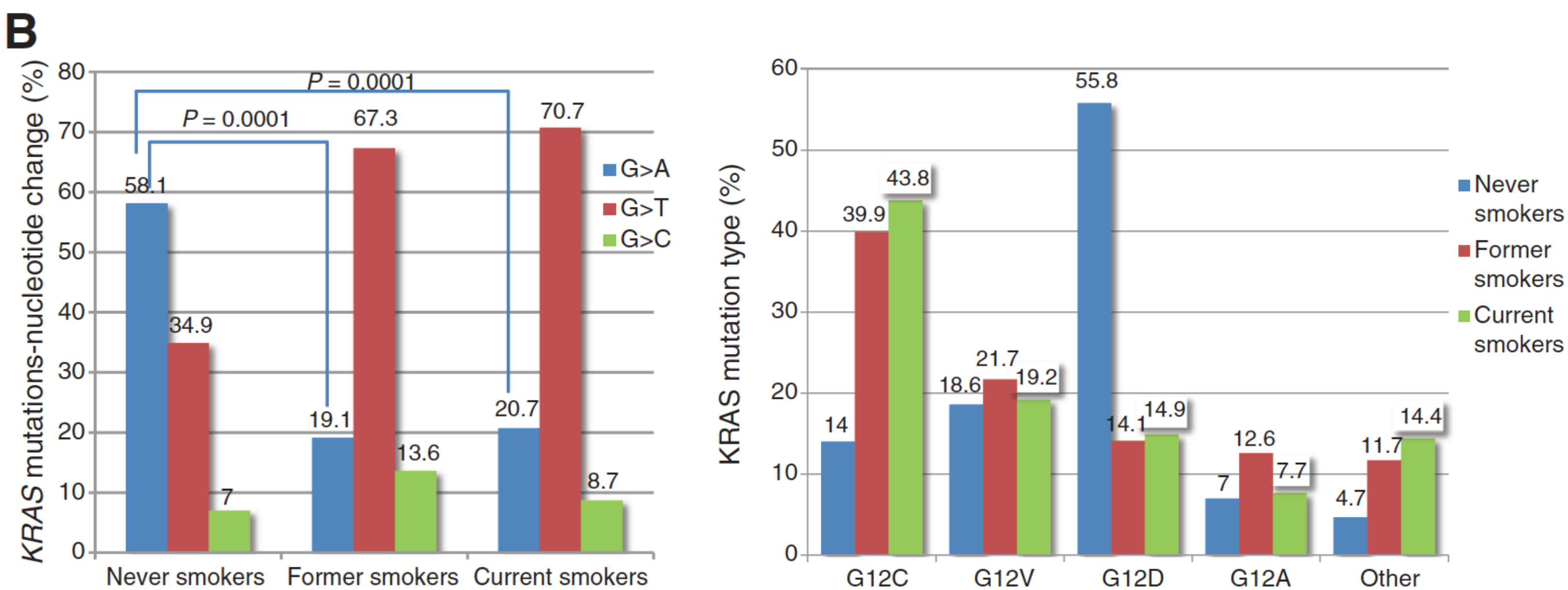
**Prior et al. Cancer Res (2012) 72:2457-67**

## What Can we Learn from Clinical Data in Specific Cancer Types Regarding How Critical it is in These Tumors and What Benefit Might Accrue By Successful Targeted Therapy?

- Lung Cancer
- Colorectal Cancer
- Pancreatic Cancer
- Leukemia (AML)

# Lung Cancer: RAS Mutation Type Related to Smoking History

N = 670 patients with KRAS mutations in lung cancer



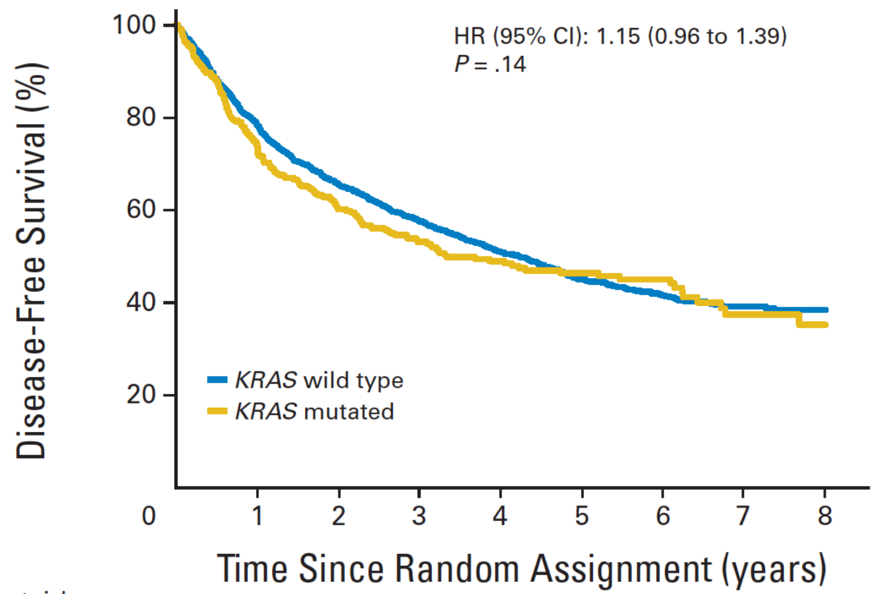

Mutations in 90% occur at codon 12, typically a G>T transversion, KRas G12C, a type induced by tobacco smoke

**Dogan S et al., Clin Cancer Res 18: 6169, 2012**

# Lung Cancer: KRAS Has NO Prognostic Value

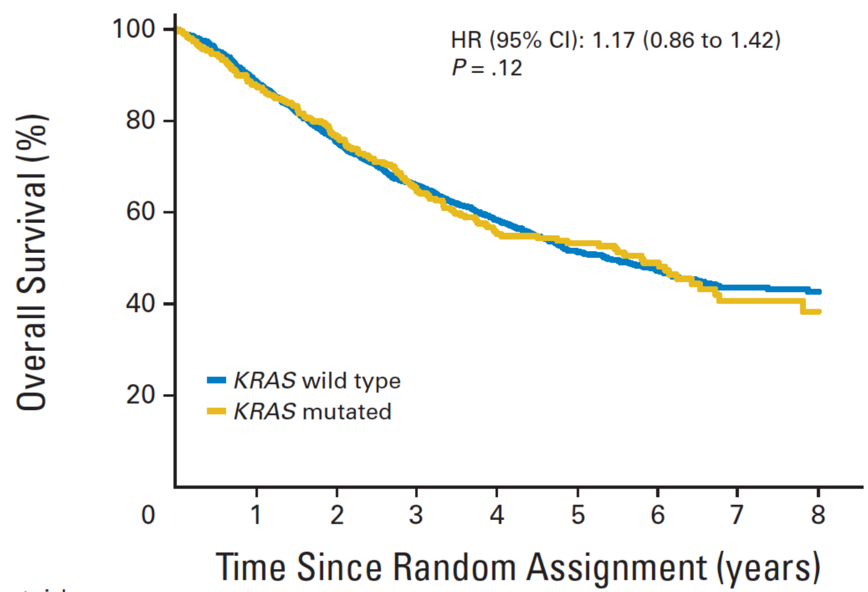
## Lung Adjuvant Cisplatin Evaluation Database:
## 1,543 patients; 300 with KRAS mutations

**Disease-free Survival**




| No. at risk | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *KRAS* wild type | 1,243 | 973 | 802 | 646 | 496 | 345 | 208 | 118 | 66 |
| *KRAS* mutated | 300 | 223 | 179 | 137 | 104 | 77 | 54 | 23 | 14 |

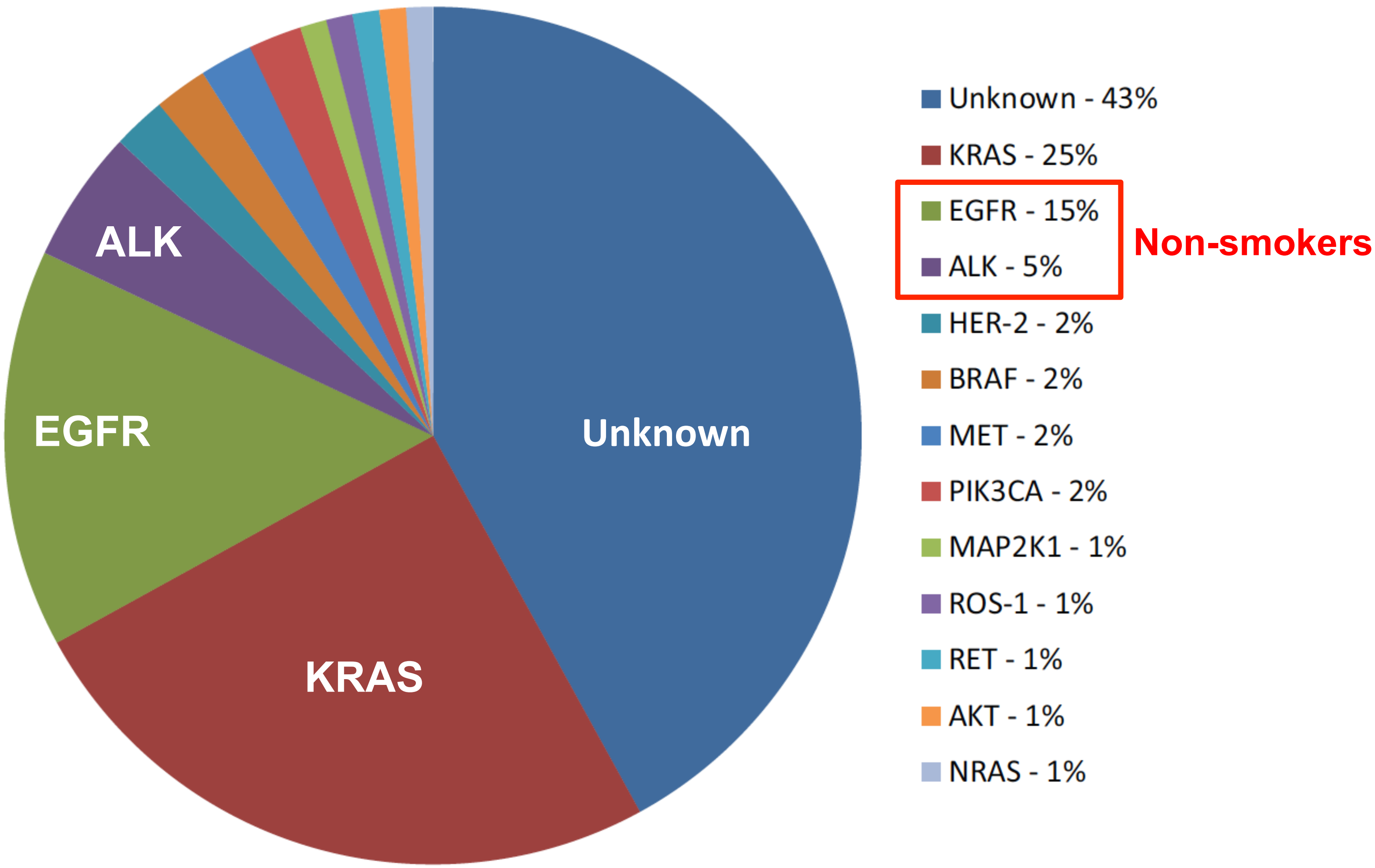
**Overall Survival**




| No. at risk | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *KRAS* wild type | 1,243 | 1,100 | 920 | 735 | 558 | 391 | 235 | 127 | 71 |
| *KRAS* mutated | 300 | 263 | 227 | 172 | 122 | 92 | 61 | 26 | 15 |

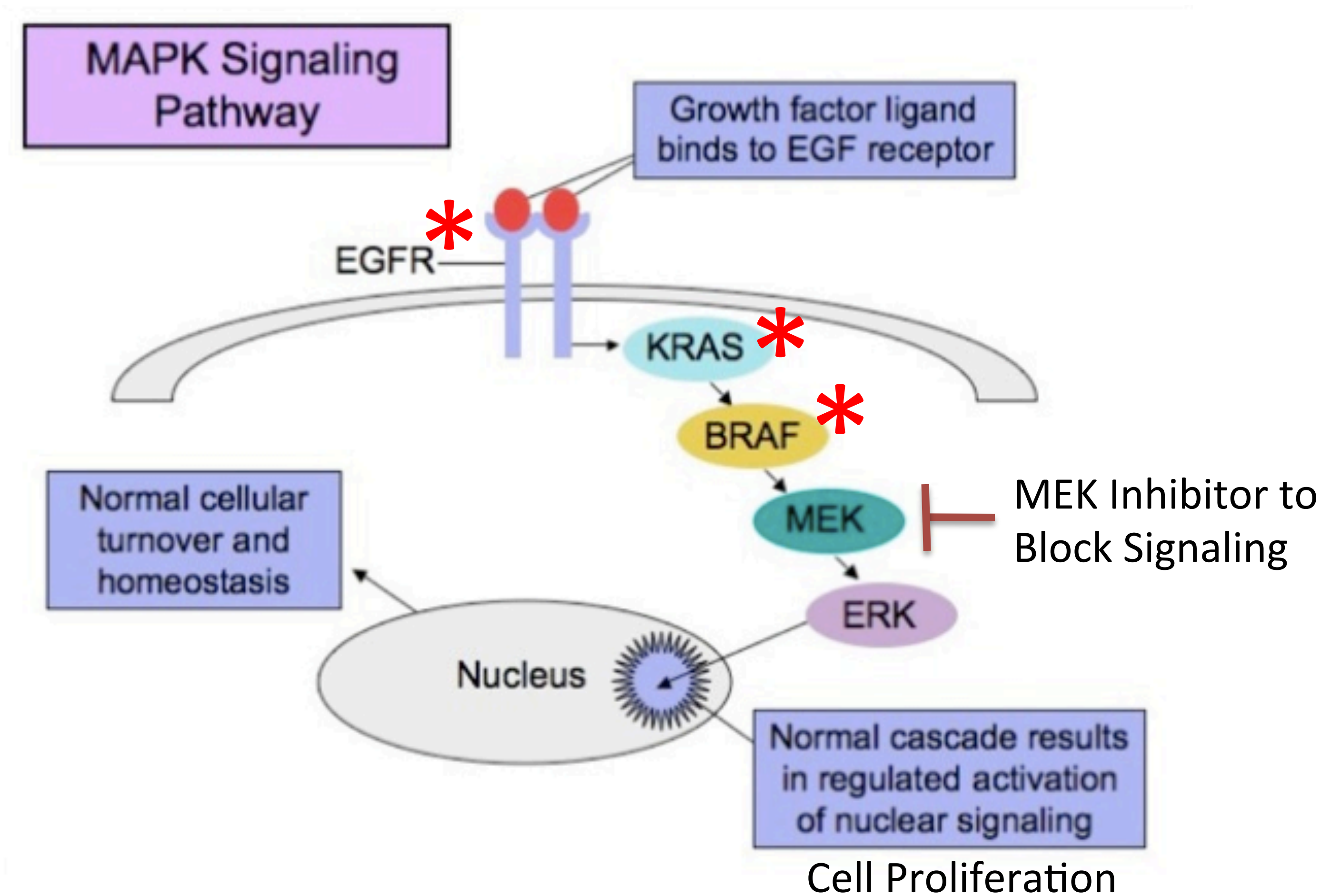
**Shepherd FA et al., J Clin Oncol 31: 2173, 2013**

# Lung Cancer:
# Might RAS be a Useful Therapeutic Target in Lung Cancer, Equivalent to EGFR and ALK?



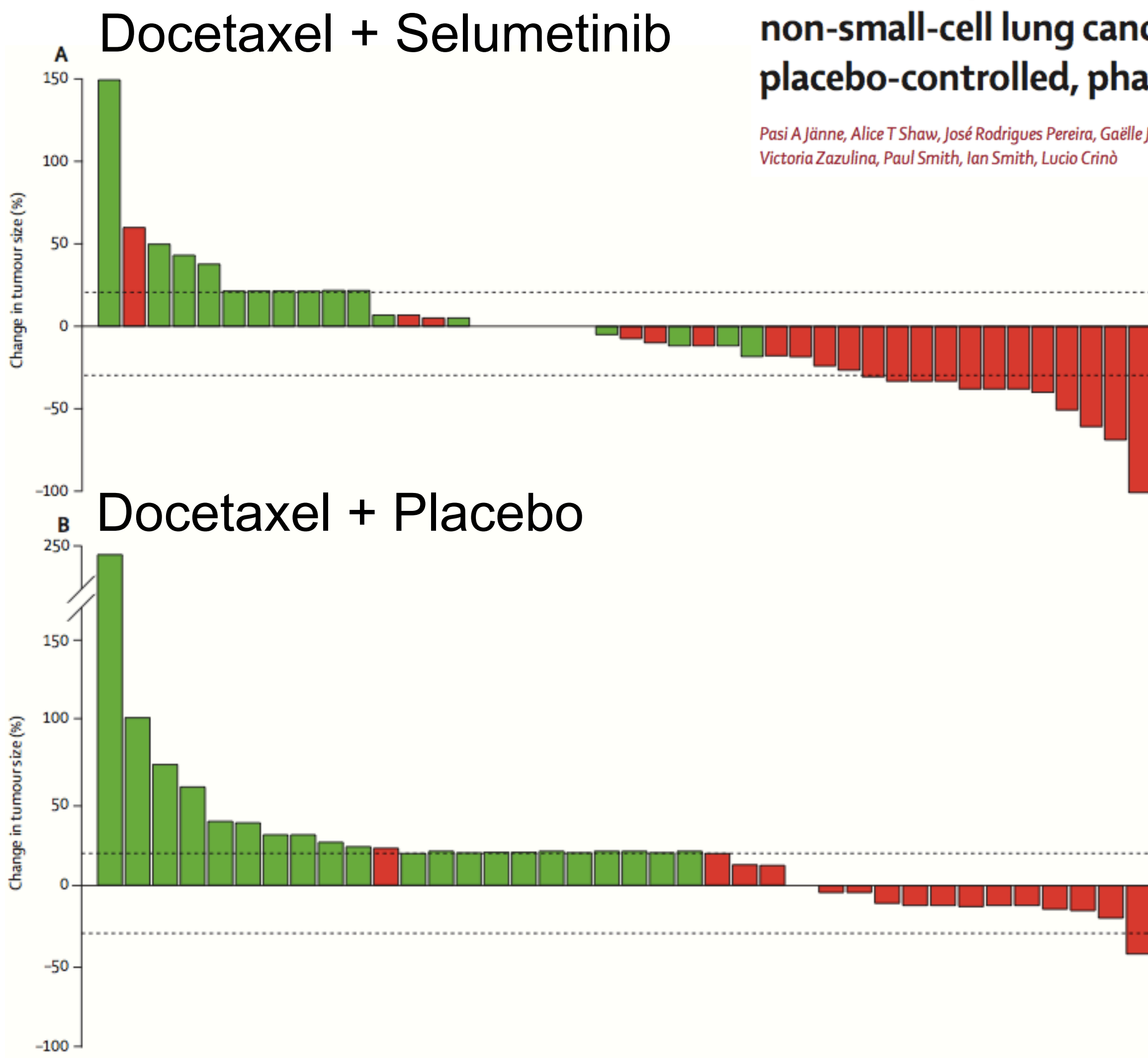

**Korpanty GJ et al, Frontiers in Oncology (2014) 4:204**

# Epidermal Growth Factor Pathway Activated in Lung Cancer at Multiple Levels: EGFR, RAS, BRAF



**Selumetinib plus docetaxel for *KRAS*-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study**

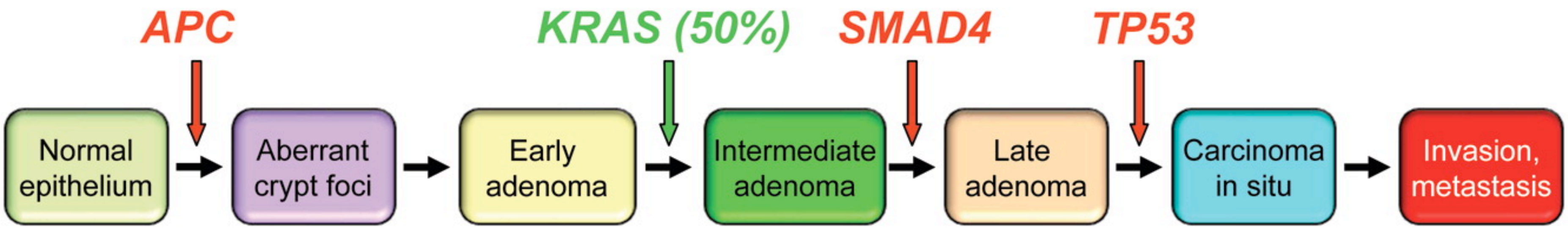
Pasi A Jänne, Alice T Shaw, José Rodrigues Pereira, Gaëlle Jeannin, Johan Vansteenkiste, Carlos Barrios, Fabio Andre Franke, Lynda Grinsted, Victoria Zazulina, Paul Smith, Ian Smith, Lucio Crinò




gure 3: Change in tumour size at 12 weeks

Median Progression-Free Survival:
5.3 vs. 2.1 months

# Colorectal Cancer: Data for the importance of KRAS are mixed: KRAS Mutations ARE NOT Initiating Events in Carcinogenesis




**How important are mutations that arise later to a cancer?**
**Uncertain**
**How vulnerable is a cancer cell harboring such a mutation to an inhibitor of such mutations?**
**Uncertain**

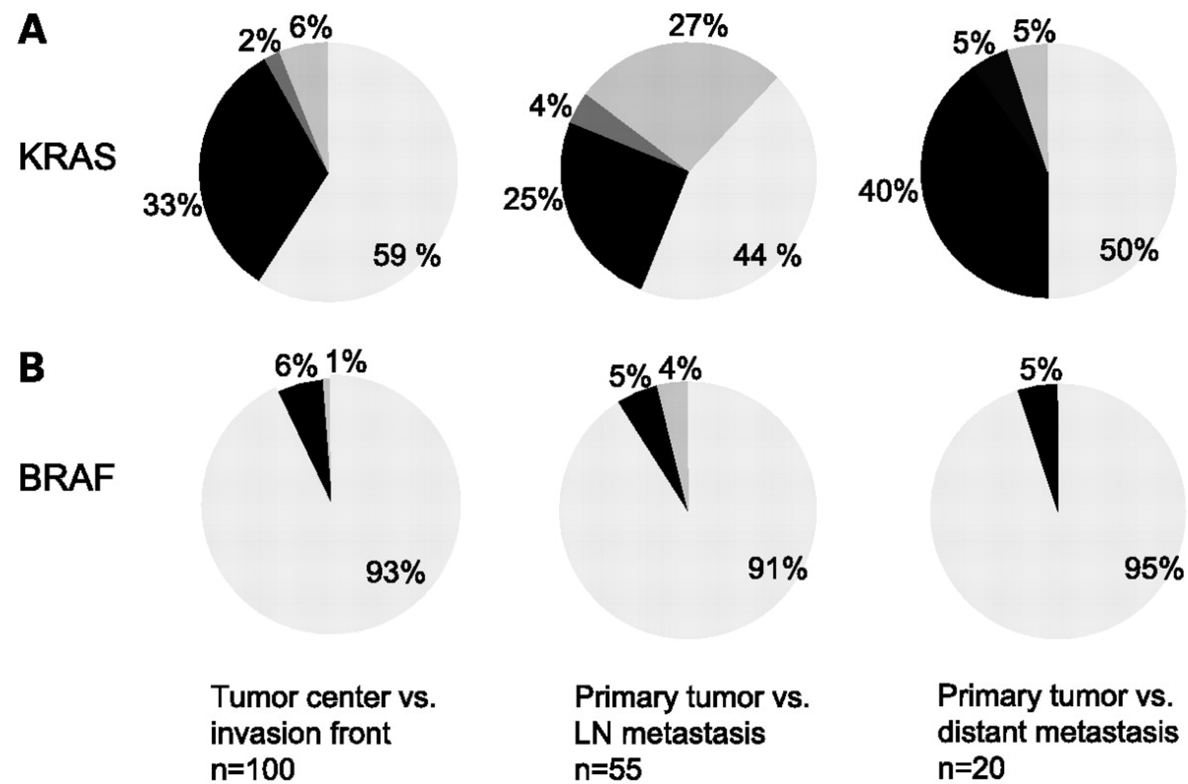
# Evidence KRAS may not be initiating (indispensable) mutation: Heterogeneous distribution of KRAS, BRAF, and PIK3CA mutations in primary tumors, lymph node, and distant metastases of colorectal cancer.

WT → WT

MUT → MUT

WT → MUT*

MUT → WT*




*Further sampling revealed KRAS mutations in primary tumor or in LN metastasis

Clinical Cancer Research

Baldus S E et al. Clin Cancer Res 2010;16:790-799

Indirect evidence from clinical trials with antibodies targeting the EGFR suggests RAS is important in colorectal cancer

Cetuximab and Panitumumab

# The FDA approved cetuximab for colorectal cancer in 2004

**FDA NEWS RELEASE**

FOR IMMEDIATE RELEASE
P04-20
February 12, 2004

Media Inquiries: 301-827-6242
Consumer Inquiries: 888-INFO-FDA

**FDA Approves Erbitux for Colorectal Cancer**

FDA today approved Erbitux (cetuximab) to treat patients with advanced colorectal cancer that has spread to other parts of the body. Erbitux is the first monoclonal antibody approved to treat this type of cancer and is indicated as a combination treatment to be given intravenously with irinotecan, another drug approved to fight colorectal cancer, or alone if patients cannot tolerate irinotecan.

Erbitux was approved under FDA's accelerated approval program, which allows FDA to approve products for cancer and other serious or life-threatening diseases based on early evidence of a product's effectiveness. Although treatment with Erbitux has not been shown to extend patients' lives, it was shown to shrink tumors in some patients and delay tumor growth, especially when used as a combination treatment.

Erbitux is a genetically engineered version of a mouse antibody that contains both human and mouse components. (Antibodies in the body are substances produced by the immune system to fight foreign substances.) It can be produced in large quantities in the laboratory. This new monoclonal antibody is believed to work by targeting a natural protein called "epidermal growth factor receptor" (EGFR) on the surface of cancer cells, interfering with their growth.

For patients with tumors that express EGFR and who no longer responded to treatment with irinotecan alone or in combination with other chemotherapy drugs, the combination treatment of Erbitux and irinotecan shrank tumors in 22.9% of patients and delayed tumor growth by approximately 4.1 months. For patients who received Erbitux alone, the tumor response rate was 10.8% and tumor growth was delayed by 1.5 months.

# Cetuximab did not benefit patients whose tumors harbor mutant KRAS, reported in 2008

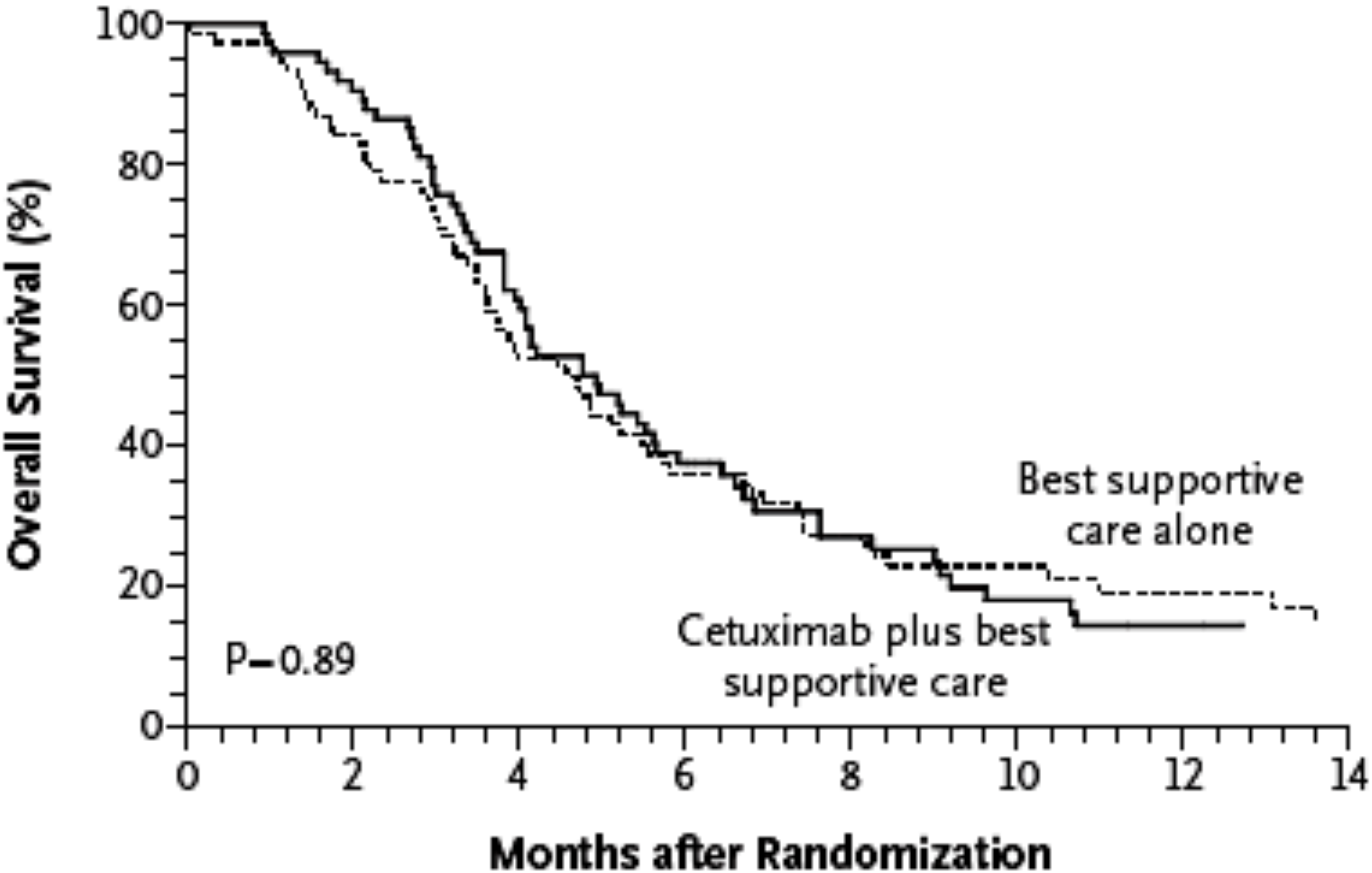
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 23, 2008 VOL. 359 NO. 17

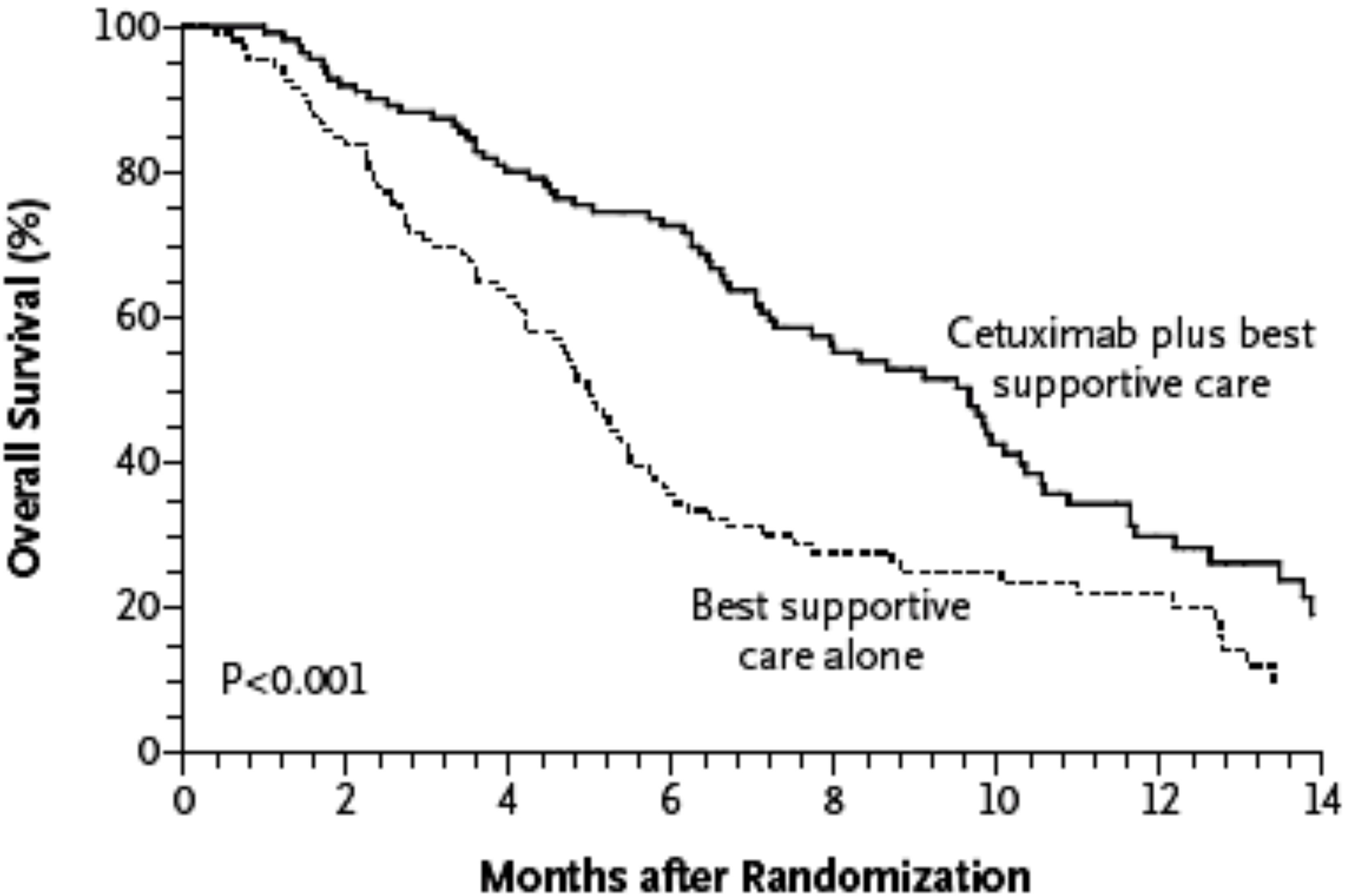
## *K-ras* Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer

Christos S. Karapetis, M.D., Shirin Khambata-Ford, Ph.D., Derek J. Jonker, M.D., Chris J. O'Callaghan, Ph.D., Dongsheng Tu, Ph.D., Niall C. Tebbutt, Ph.D., R. John Simes, M.D., Haji Chalchal, M.D., Jeremy D. Shapiro, M.D., Sonia Robitaille, M.Sc., Timothy J. Price, M.D., Lois Shepherd, M.D.C.M., Heather-Jane Au, M.D., Christiane Langer, M.D., Malcolm J. Moore, M.D., and John R. Zalcberg, M.D., Ph.D.*

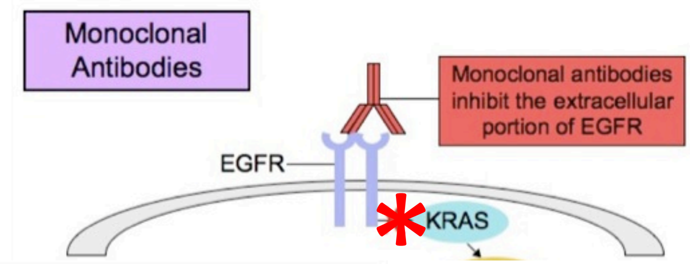
# Cetuximab vs Best Supportive Care
# Mutant K-ras: No benefit

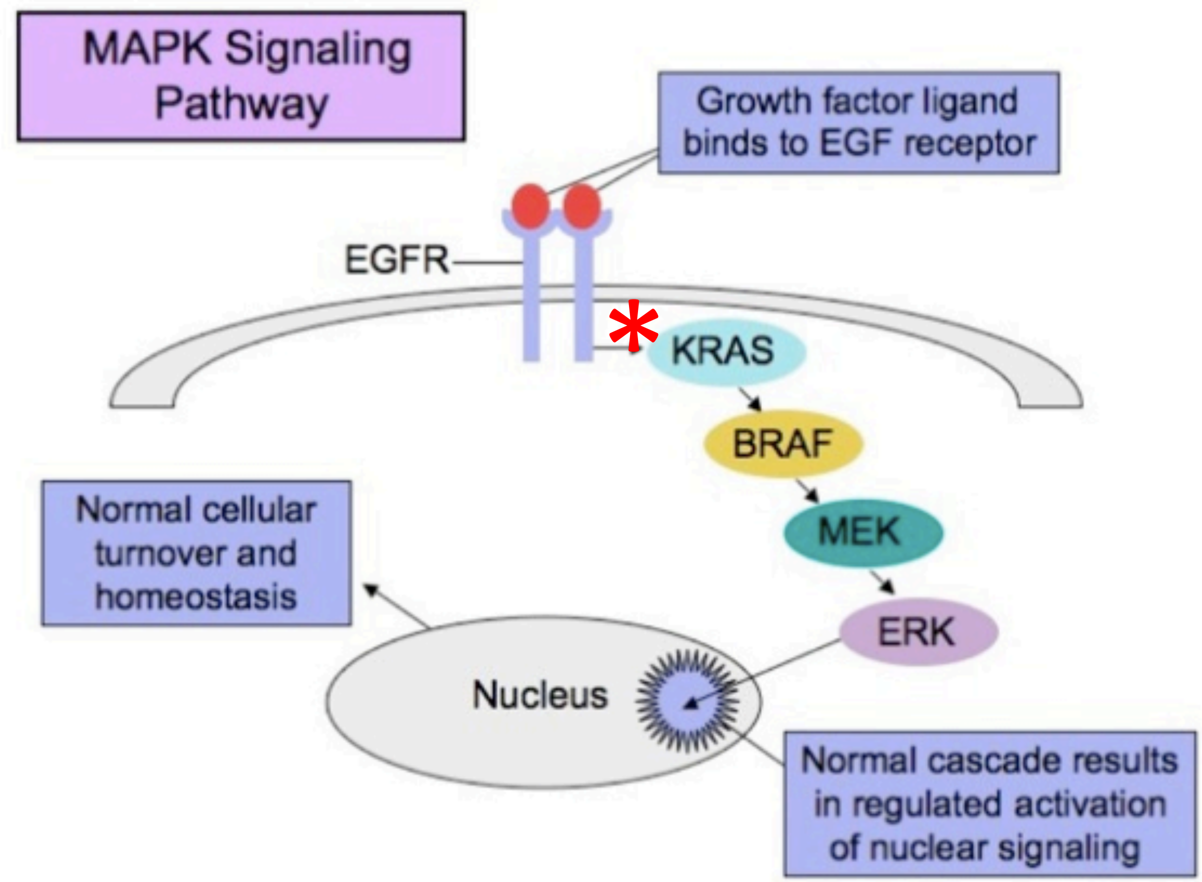


# Cetuximab vs Best Supportive Care
# Wild-type K-ras: Apparent benefit



# Constitutive RAS activation results in insensitivity to antibodies targeting the EGFR




Cell Proliferation

# ASCO issues a "Provisional Clinical Opinion" "suggesting" that KRAS testing be performed In patients with colorectal cancer before administering cetuximab in 2009

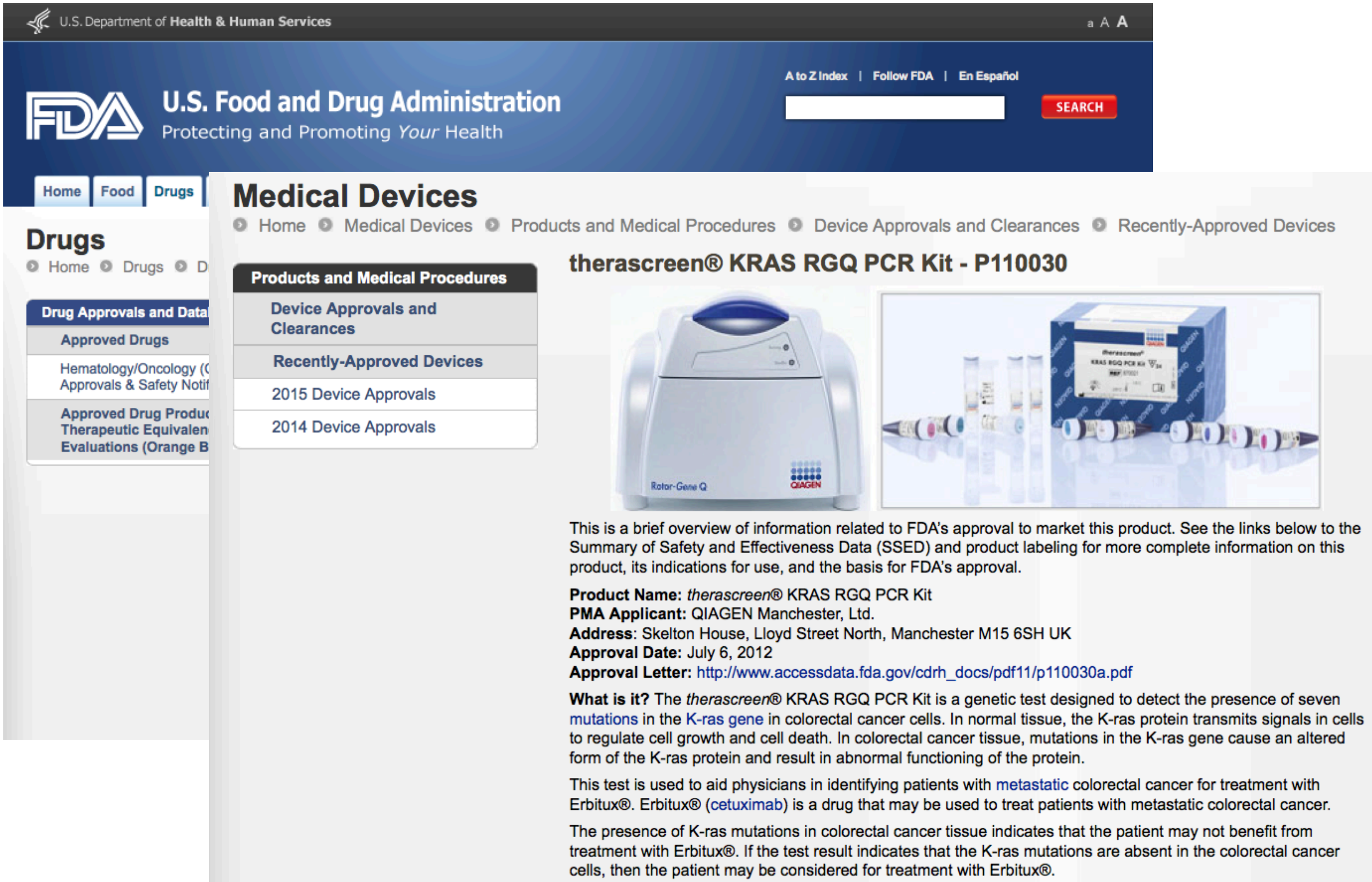
VOLUME 27 · NUMBER 12 · APRIL 20 2009

JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

## American Society of Clinical Oncology Provisional Clinical Opinion: Testing for *KRAS* Gene Mutations in Patients With Metastatic Colorectal Carcinoma to Predict Response to Anti–Epidermal Growth Factor Receptor Monoclonal Antibody Therapy

*Carmen J. Allegra, J. Milburn Jessup, Mark R. Somerfield, Stanley R. Hamilton, Elizabeth H. Hammond, Daniel F. Hayes, Pamela K. McAllister, Roscoe F. Morton, and Richard L. Schilsky*

# KRAS mutation test: First FDA approved companion diagnostic based on a cancer-causing mutation: July 2012



U.S. Department of Health & Human Services

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- 2014 Device Approvals

### therascreen® KRAS RGQ PCR Kit - P110030

This is a brief overview of information related to FDA's approval to market this product. See the links below to the Summary of Safety and Effectiveness Data (SSED) and product labeling for more complete information on this product, its indications for use, and the basis for FDA's approval.

**Product Name:** *therascreen*® KRAS RGQ PCR Kit
**PMA Applicant:** QIAGEN Manchester, Ltd.
**Address**: Skelton House, Lloyd Street North, Manchester M15 6SH UK
**Approval Date:** July 6, 2012
**Approval Letter:** http://www.accessdata.fda.gov/cdrh_docs/pdf11/p110030a.pdf

**What is it?** The *therascreen*® KRAS RGQ PCR Kit is a genetic test designed to detect the presence of seven mutations in the K-ras gene in colorectal cancer cells. In normal tissue, the K-ras protein transmits signals in cells to regulate cell growth and cell death. In colorectal cancer tissue, mutations in the K-ras gene cause an altered form of the K-ras protein and result in abnormal functioning of the protein.

This test is used to aid physicians in identifying patients with metastatic colorectal cancer for treatment with Erbitux®. Erbitux® (cetuximab) is a drug that may be used to treat patients with metastatic colorectal cancer.

The presence of K-ras mutations in colorectal cancer tissue indicates that the patient may not benefit from treatment with Erbitux®. If the test result indicates that the K-ras mutations are absent in the colorectal cancer cells, then the patient may be considered for treatment with Erbitux®.

**Companion Diagnostics: Developing Precision Medicine in a Global World. Rubin EH et al. Clin Cancer Res. 20: 1419, 2014**

# *therascreen® KRAS RGQ PCR Kit*
# *QIAGEN Manchester Ltd*

FDA Approval July 6, 2012

II. **INDICATIONS FOR USE**

The therascreen® KRAS RGQ PCR Kit is a real-time qualitative PCR assay used on the Rotor-Gene Q MDx instrument for the detection of seven somatic mutations in the human KRAS oncogene, using DNA extracted from **formalin-fixed paraffin-embedded (FFPE), colorectal cancer (CRC) tissue**. The therascreen® KRAS RGQ PCR Kit is intended to **aid in the identification of CRC patients for treatment with Erbitux (cetuximab) and Vectibix® (panitumumab)** based on a KRAS no mutation detected test result.

| Mutation | Base Change |
|---|---|
| GLY12ALA (G12A) | GGT>GCT |
| GLY12ASP (G12D) | GGT>GAT |
| GLY12ARG (G12R) | GGT>CGT |
| GLY12CYS (G12C) | GGT>TGT |
| GLY12SER (G12S) | GGT>AGT |
| GLY12VAL (G12V) | GGT>GTT |
| GLY13ASP (G13D) | GGC>GAC |

# Cetuximab in Combination with Folfiri / Therascreen

U. S. Food and Drug Administration granted approval

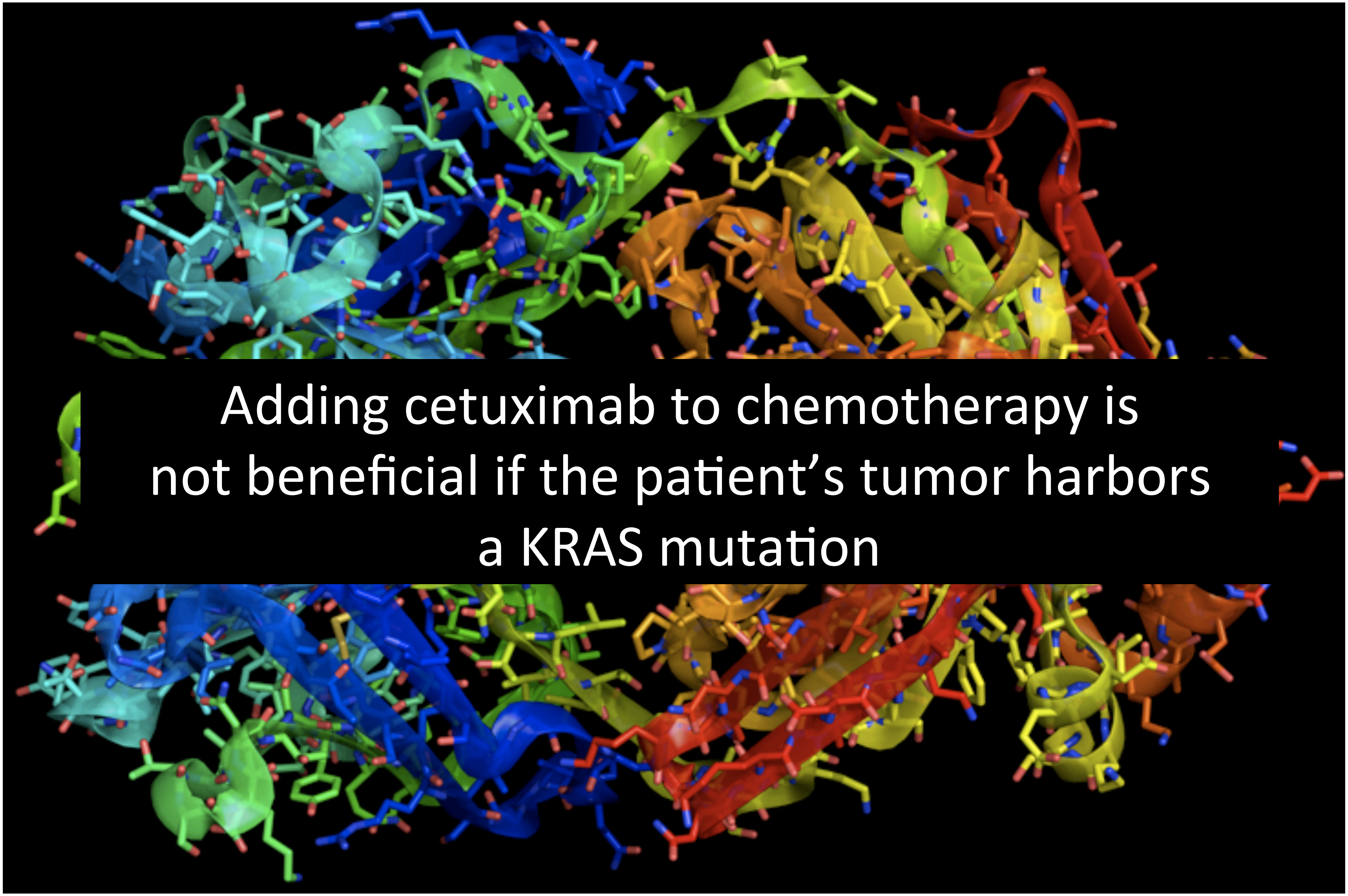
July 6, 2012

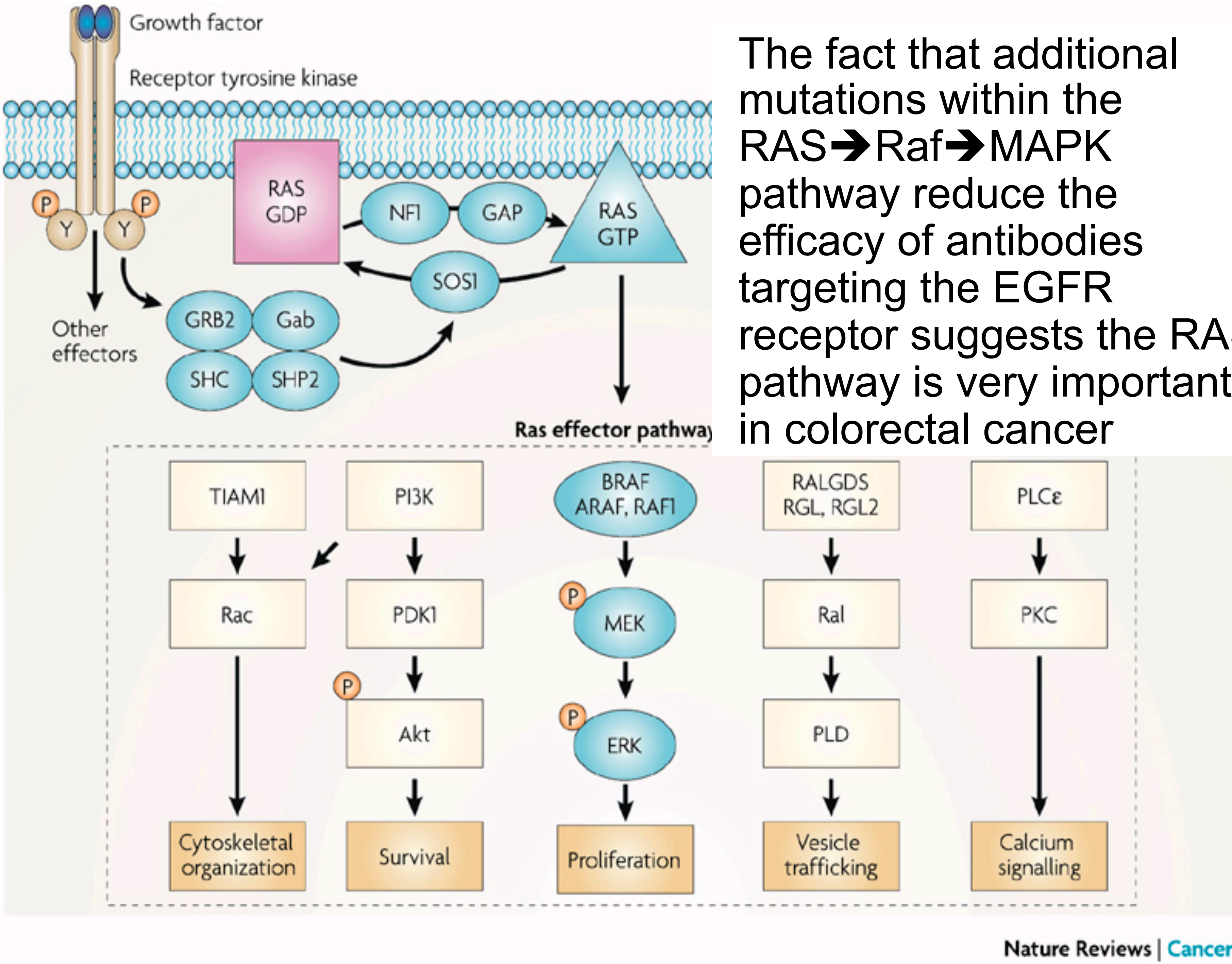
Three clinical trials in patients with metastatic colorectal cancer. Note that the therascreen assay detects MUTATIONS, not Wild-Type sequence:

CRYSTAL trial: 1,214 patients
EGFR-positive tumors, no prior therapy – FOLFIRI +/- cetuximab
1,079 89% of patients had KRAS wild-type, 676, 37% had KRAS mutant tumors
Cetuximab improved OS from 19.5 to 23.5 months, response rate from 39% to 57%
**Improvement seen ONLY in the patients with wild-type tumors**

OPUS: 337 patients
EGFR positive tumors, no prior therapy - FOLFOX4 +/- cetuximab
Cetuximab improved OS from 18.5 to 22.8 months, response rate from 34% to 57%
**Improvement seen ONLY in the patients with wild-type tumors**

http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm310933.htm


Adding cetuximab to chemotherapy is
not beneficial if the patient's tumor harbors
a KRAS mutation



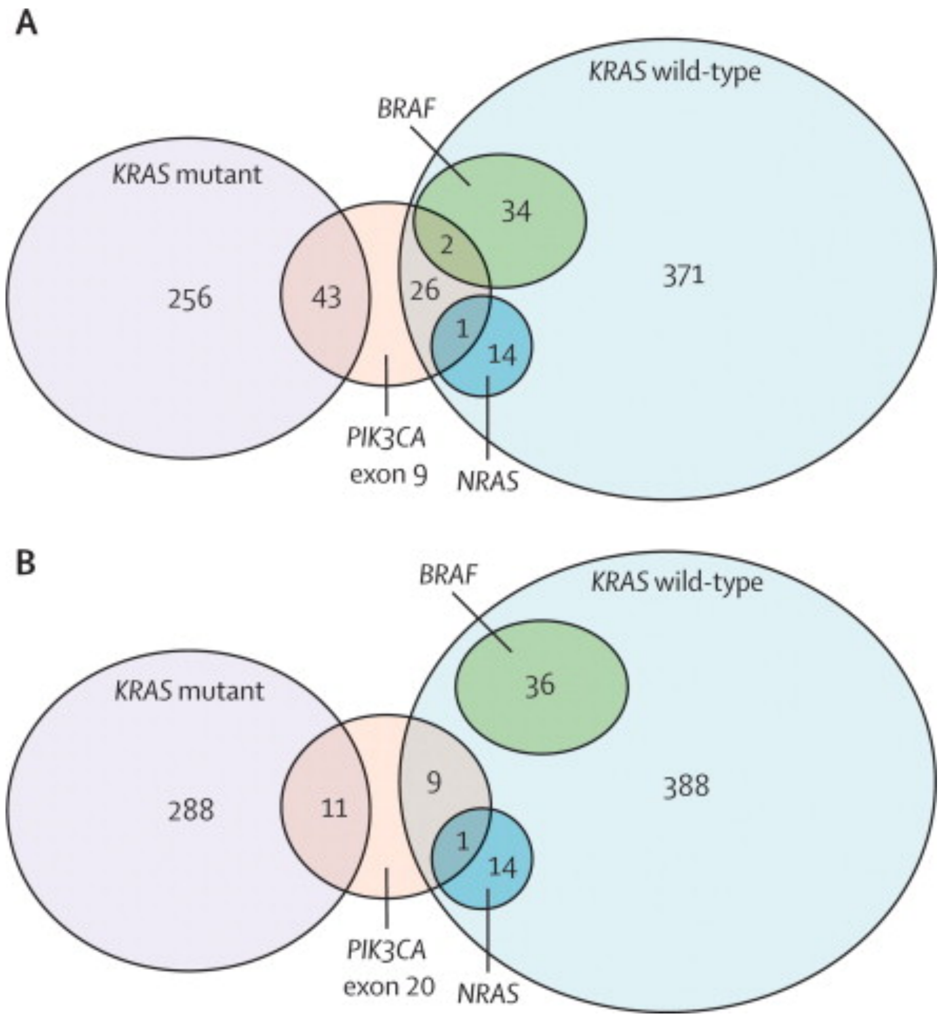

The fact that additional mutations within the RAS→Raf→MAPK pathway reduce the efficacy of antibodies targeting the EGFR receptor suggests the RAS pathway is very important in colorectal cancer

# Patients whose tumors harbor BRAF mutations have no benefit from cetuximab

| Variable | Wild-Type *BRAF* | Mutated *BRAF* | P Value | |
|---|---|---|---|---|
| No. of patients | | | | |
| CB group | 243 | 17 | | |
| CBC group | 231 | 28 | | |
| Median progression-free survival (mo) | | | | |
| CB group | 12.2 | 5.9 | | ← Control |
| CBC group | 10.4 | 6.6 | | ← Cetuximab |
| Median overall survival (mo) | | | | |
| CB group | 24.6 | 15.0 | | ← Control |
| CBC group | 21.5 | 15.2 | | ← Cetuximab |
| Response rate (%) | | | | |
| CB group | 50 | 35 | | ← Control |
| CBC group | 48 | 39 | | ← Cetuximab |

**Tol et al, N Engl J Med. 2009;361:98-9**

# Effects of *KRAS, BRAF, NRAS*, and *PIK3CA* mutations on the efficacy of cetuximab plus chemotherapy ….in mCRC: a retrospective consortium analysis

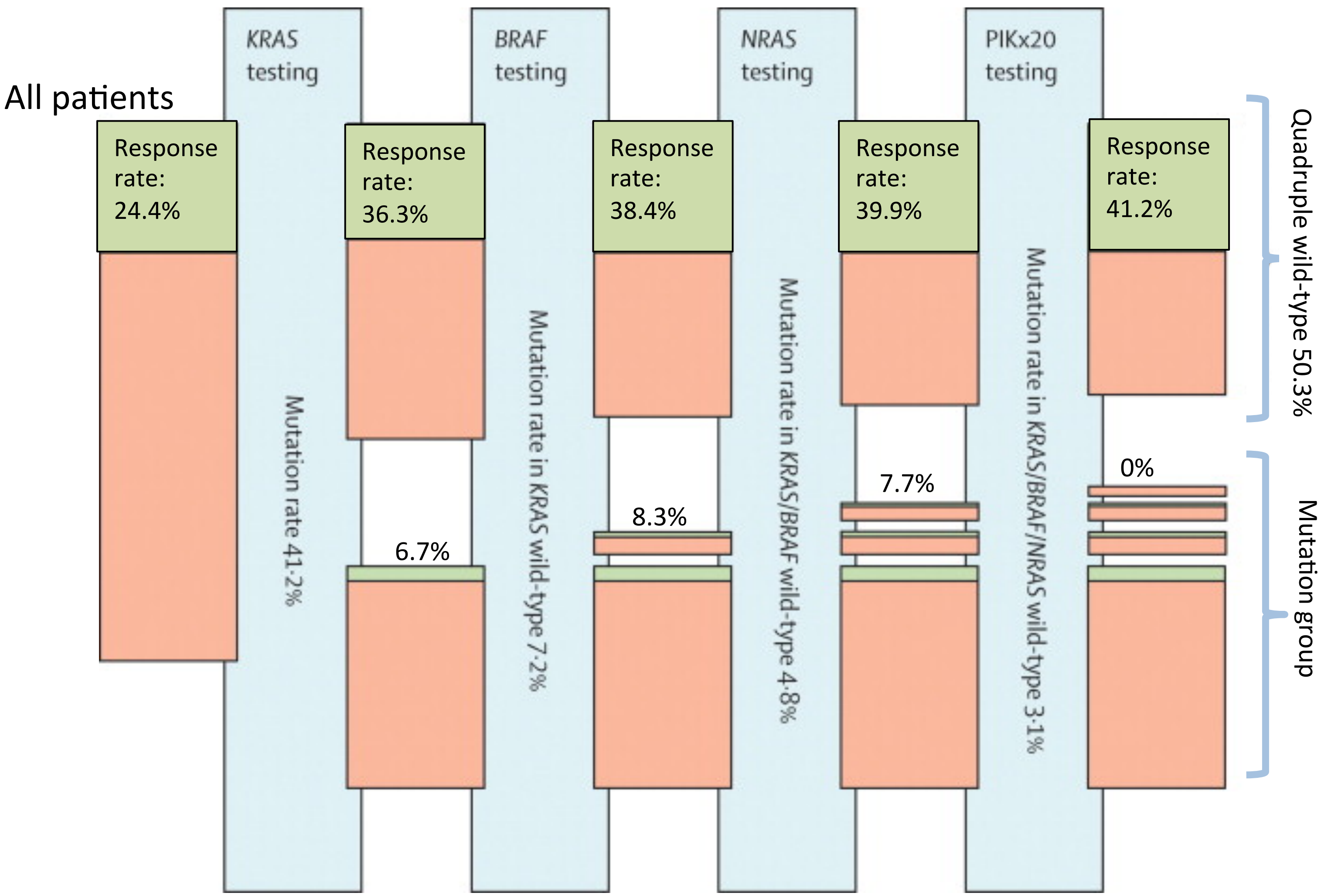



**649 patients treated with cetuximab plus chemotherapy

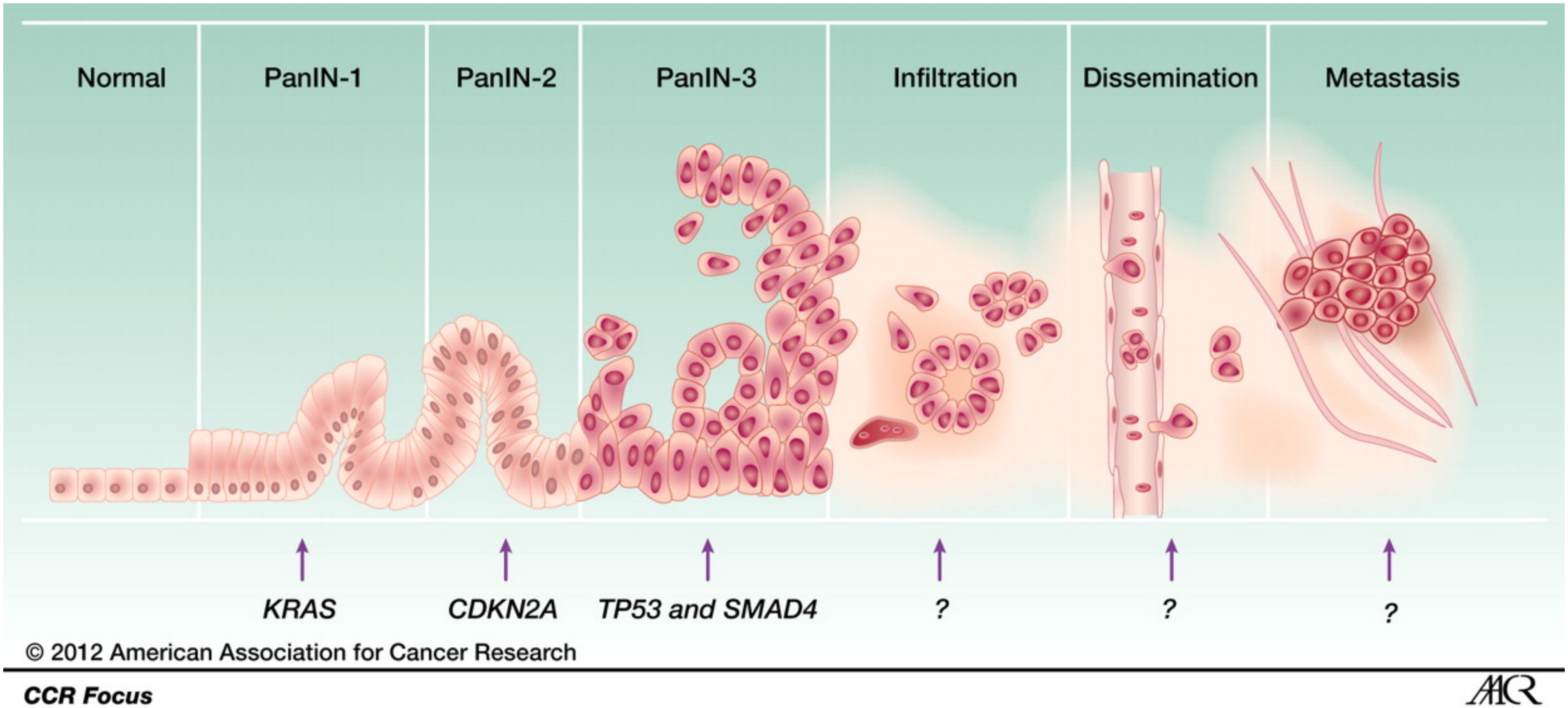
***KRAS and BRAF, KRAS and NRAS mutations were mutually exclusive

**De Roock W., Claes B., et al. The Lancet Oncology, 11:753 – 762, 2010**

# Effects of *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutations on the efficacy of cetuximab plus chemotherapy ….in mCRC: defining mutation spectrum improves response

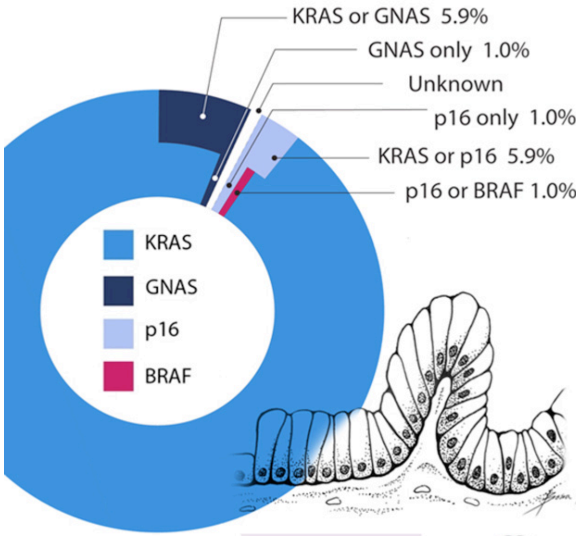


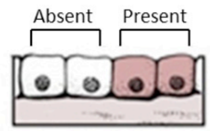
# Pancreatic Cancer: Model of progression from a normal cell to metastatic pancreatic cancer.




Clinical Cancer Research

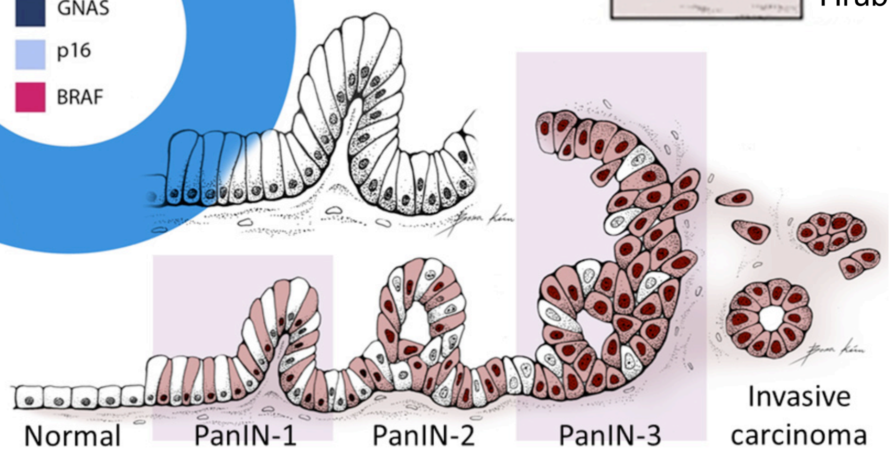
Iacobuzio-Donahue C A et al. Clin Cancer Res 2012;18:4257-4265

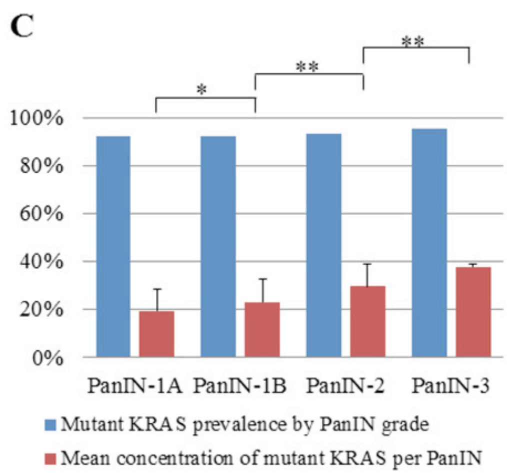


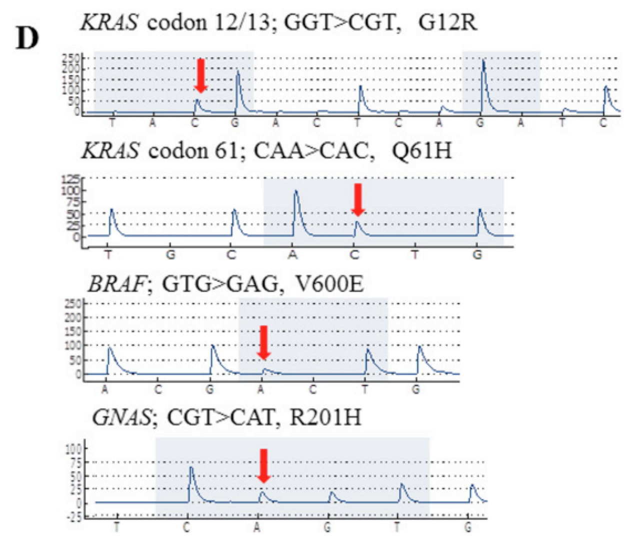




# Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia.

Kanda M1, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B, Goggins M.

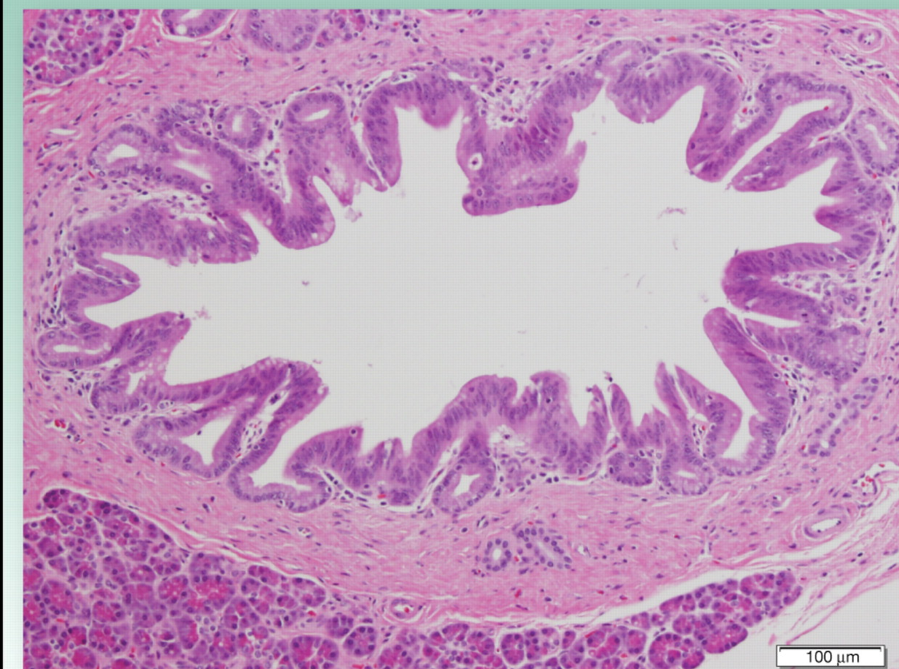



Gastroenterology 142:730-733.e9, 2012

Unlike other cancers, KRAS mutations in the earliest pancreatic intraductal lesions suggests RAS may be fundamental to pancreatic cancer oncogenesis and a proving ground for a RAS therapeutic





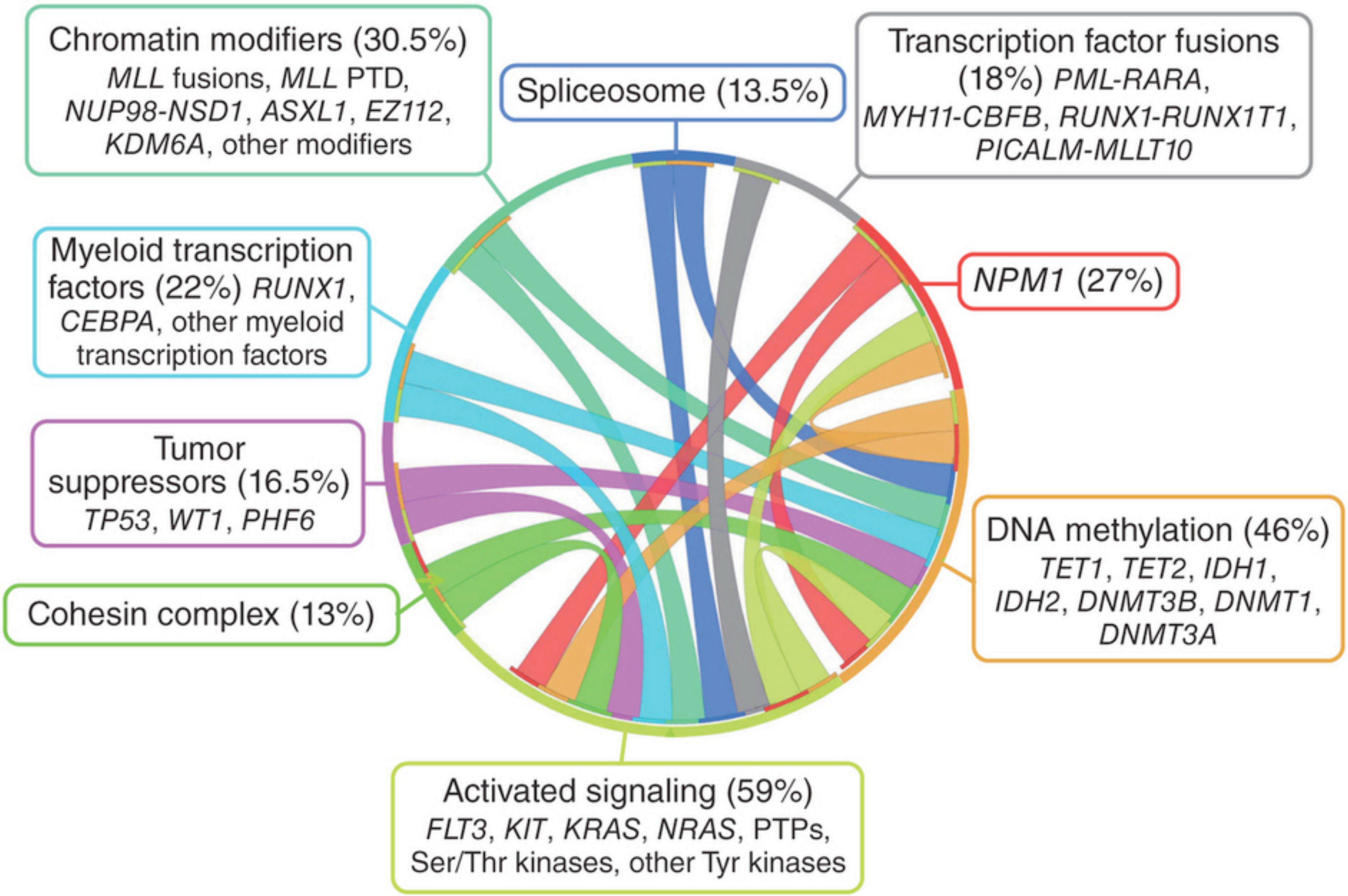

CCR Focus

Clinical Cancer Research

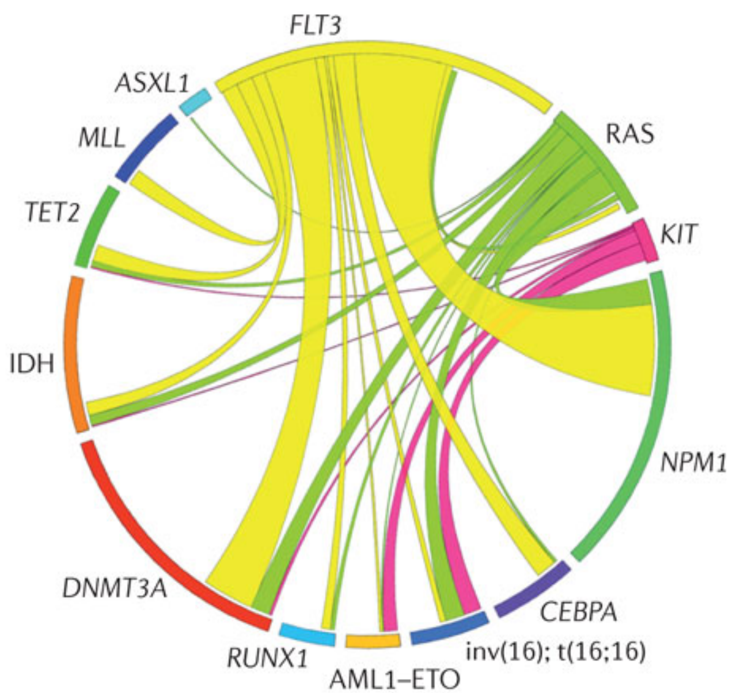
Iacobuzio-Donahue C A et al. Clin Cancer Res 2012;18:4257-4265

# Acute Myelogenous Leukemia

# Circos Diagram for Mutations in AML



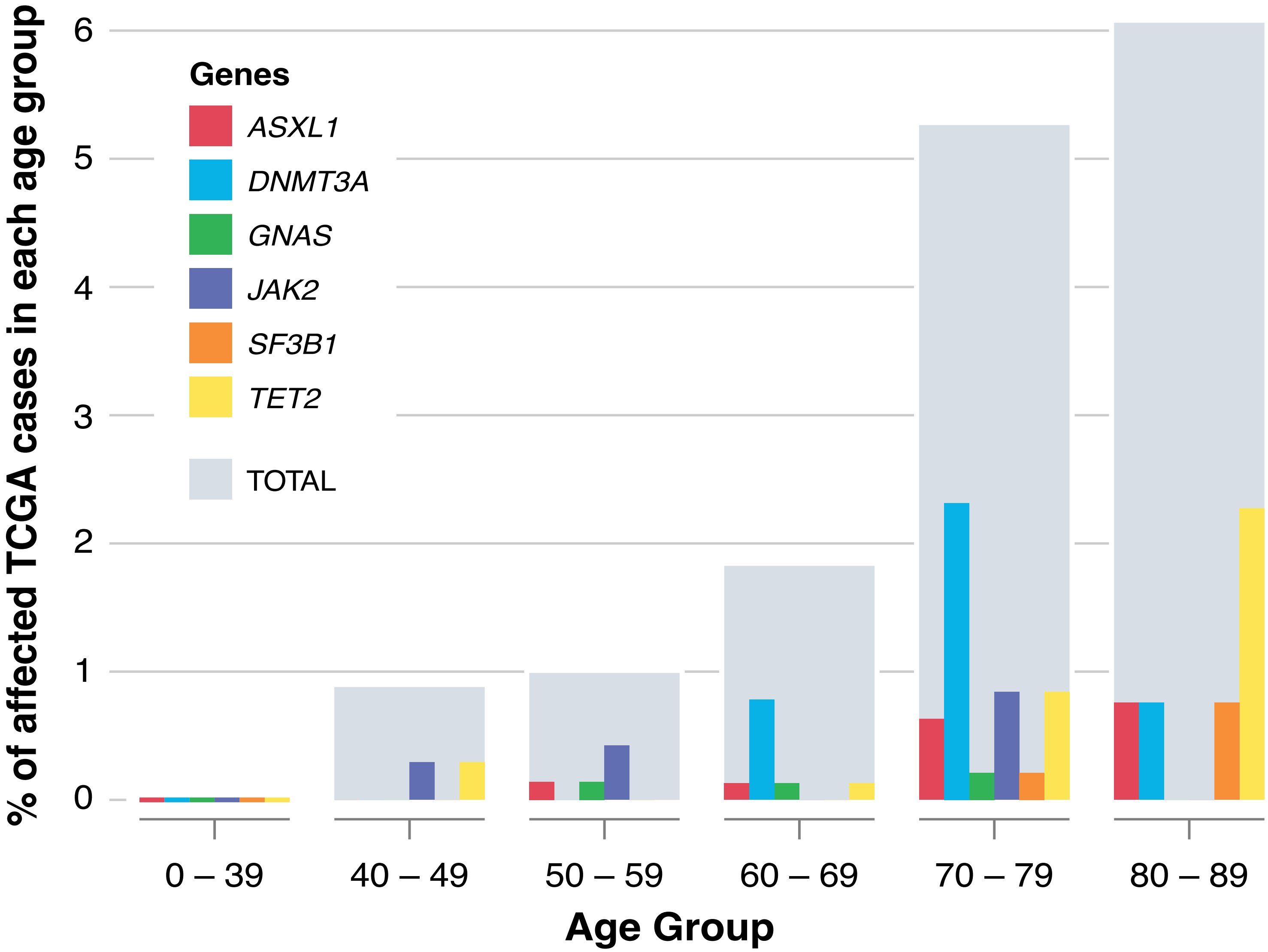
# Mutational Complexity of Acute Myeloid Leukemia (AML) in 398 Patients: Circos Diagram Depicts the Pairwise Co-occurrence of Mutations




| Gene | Overall Frequency (%) |
|---|---|
| *FLT3* (ITD, TKD) | 37 (30, 7) |
| *NPM1* | 29 |
| *DNMT3A* | 23 |
| *NRAS* | 10 |
| *CEBPA* | 9 |
| *TET2* | 8 |
| *WT1* | 8 |
| *IDH2* | 8 |
| *IDH1* | 7 |
| *KIT* | 6 |
| *RUNX1* | 5 |
| *MLL*-PTD | 5 |
| *ASXL1* | 3 |
| *PHF6* | 3 |
| *KRAS* | 2 |
| *PTEN* | 2 |
| *TP53* | 2 |
| *HRAS* | 0 |
| *EZH2* | 0 |

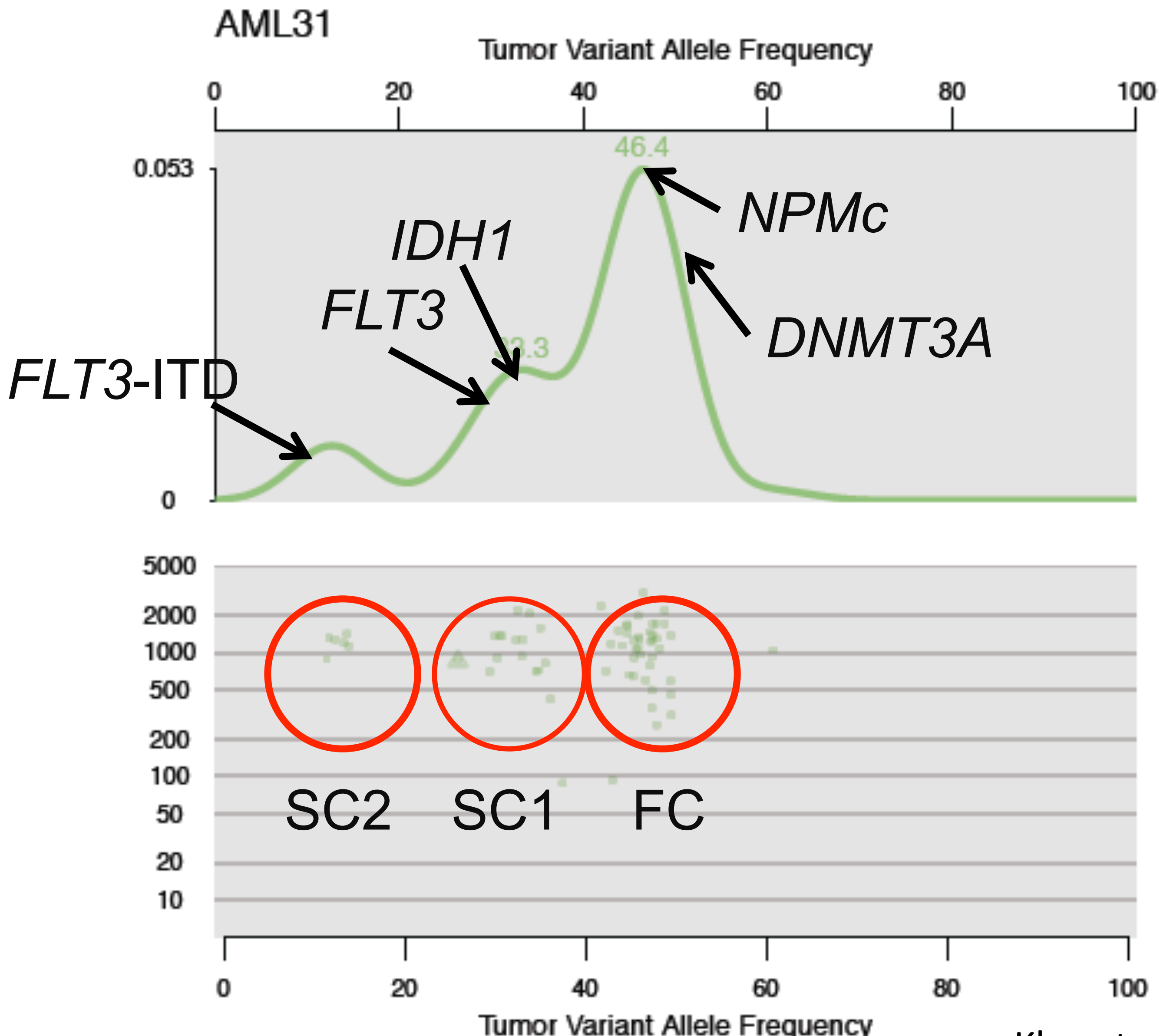
Patel JP et al. N Engl J Med 2012;366:1079-1089.

# Evidence of AML initiating mutations and clonal skewing in elderly patients' blood



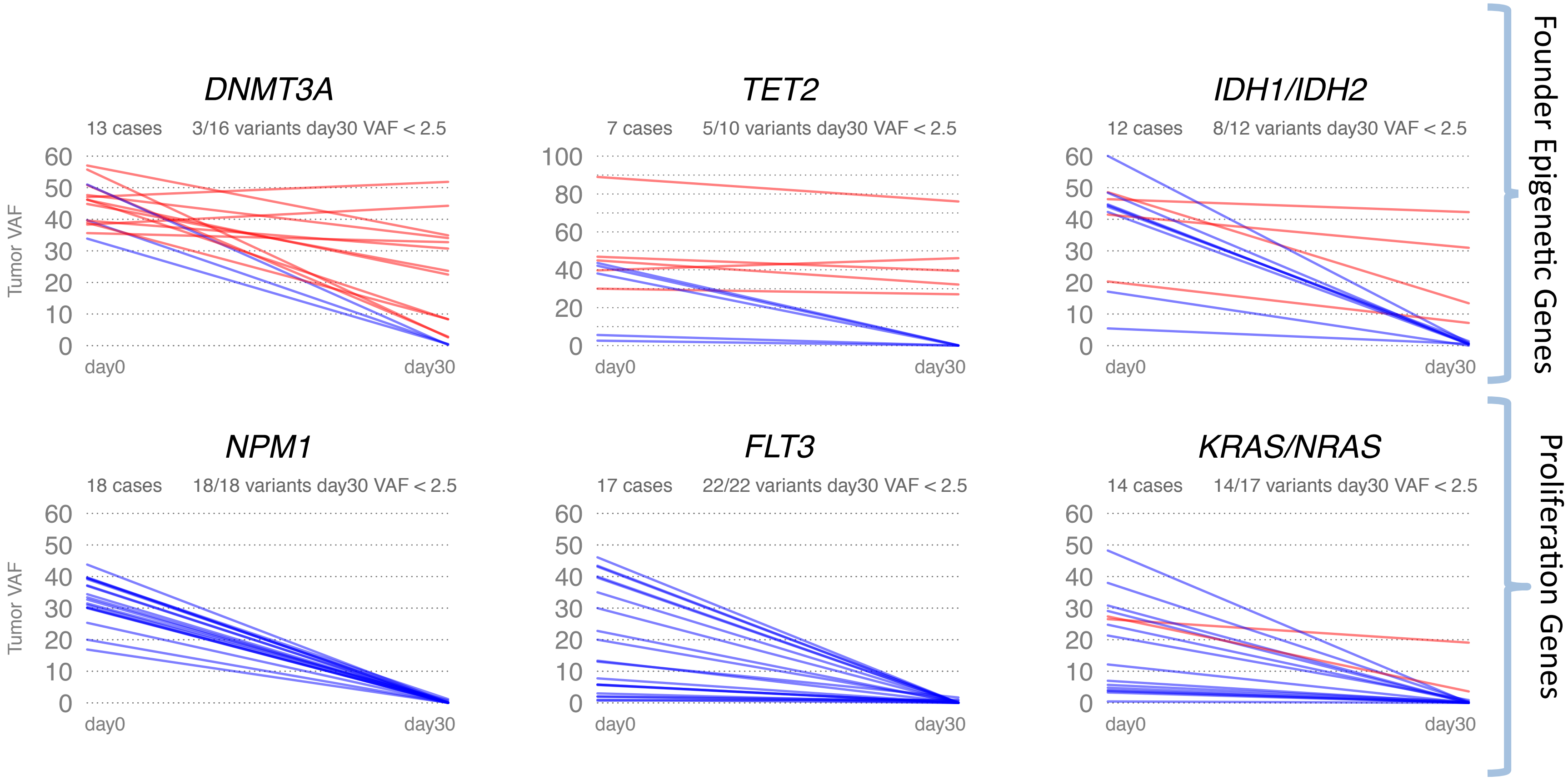

Xie et al Nature Medicine 2014

# AML 31: DNMT3A and NPM are founding mutations



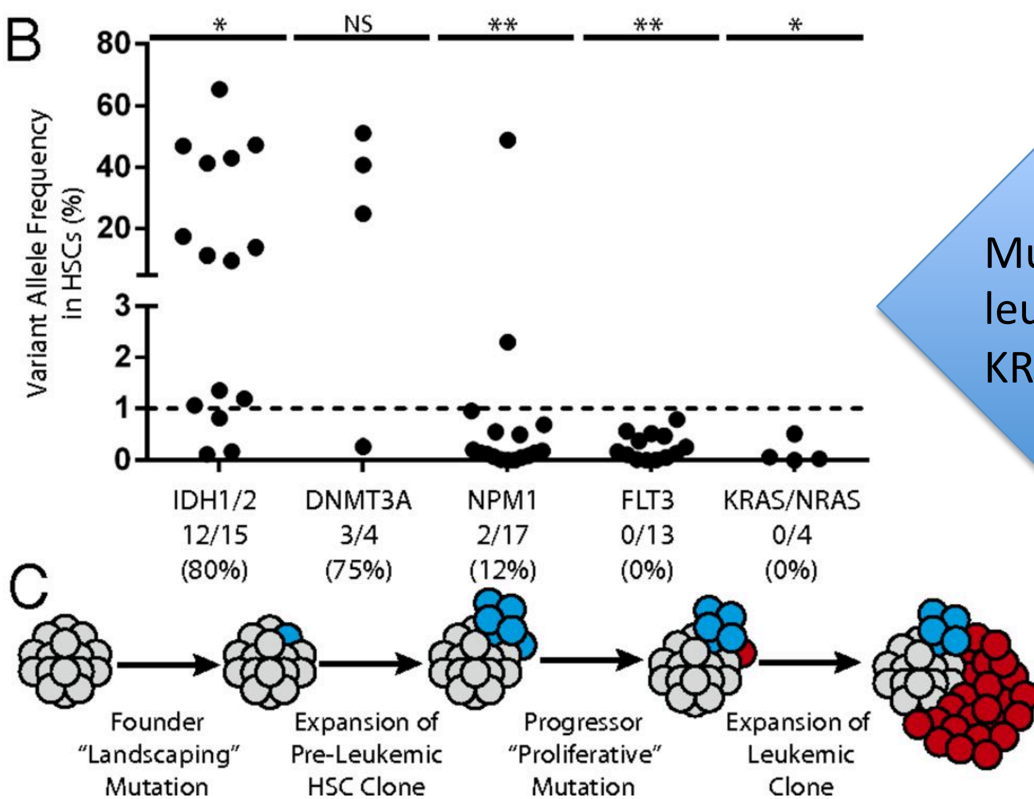

Klco et al Cancer Cell 2014

# AML: n=50, Founding mutations persist in remission




Klco, Miller, Petti, and Ley, 2015

# Mutation Acquisition in AML: preleukemic landscaping mutations followed by late proliferative mutations.




Mutations in pre-leukemic clones: KRAS is never seen

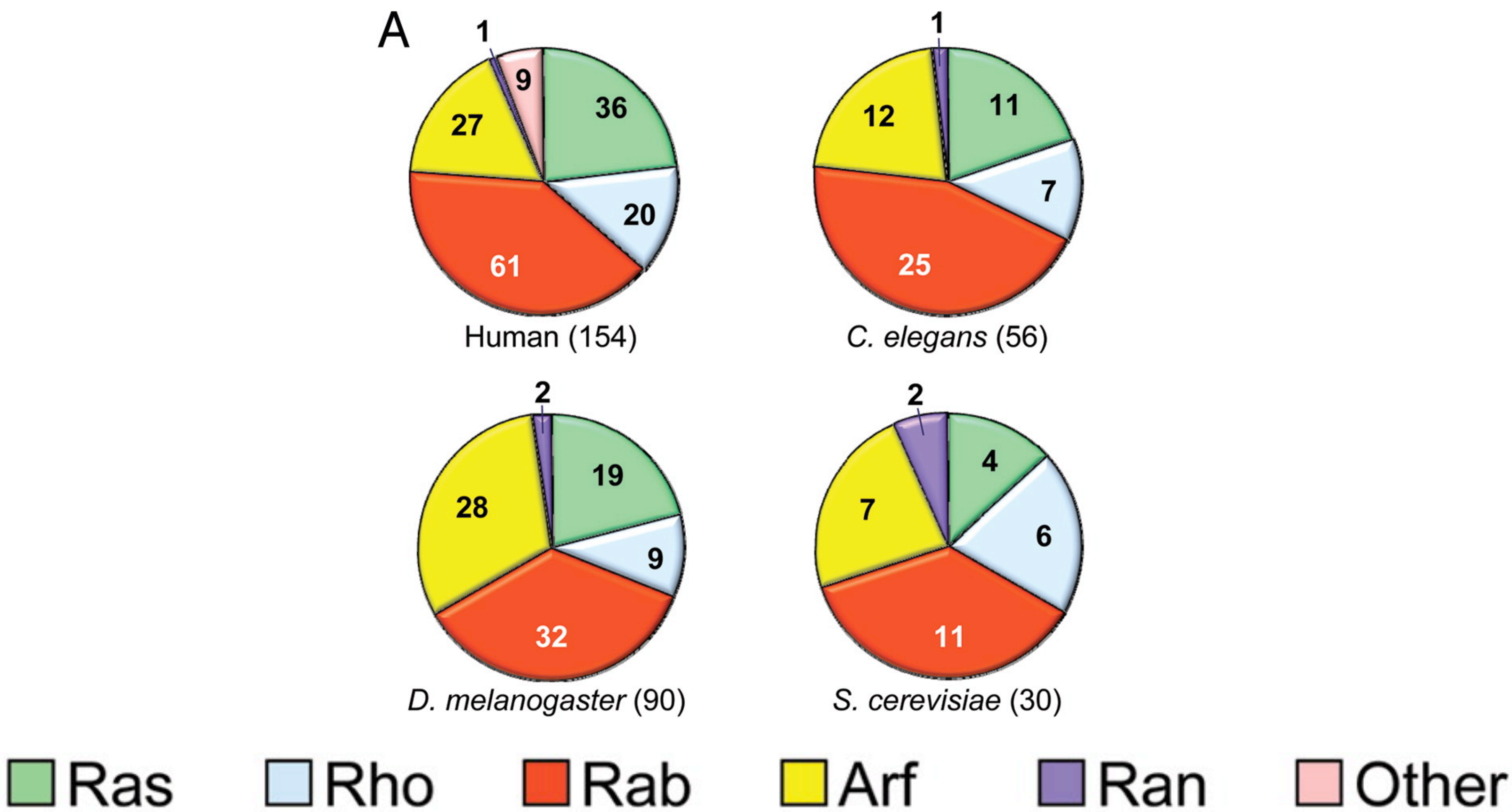
Corces-Zimmerman M R et al. PNAS 2014;111:2548-2553

# Leukemia

- Ras is not the precursor lesion
- Ras is a late mutation and a proliferative signal that drives the expansion of leukemic cells
- Inhibition not likely curative

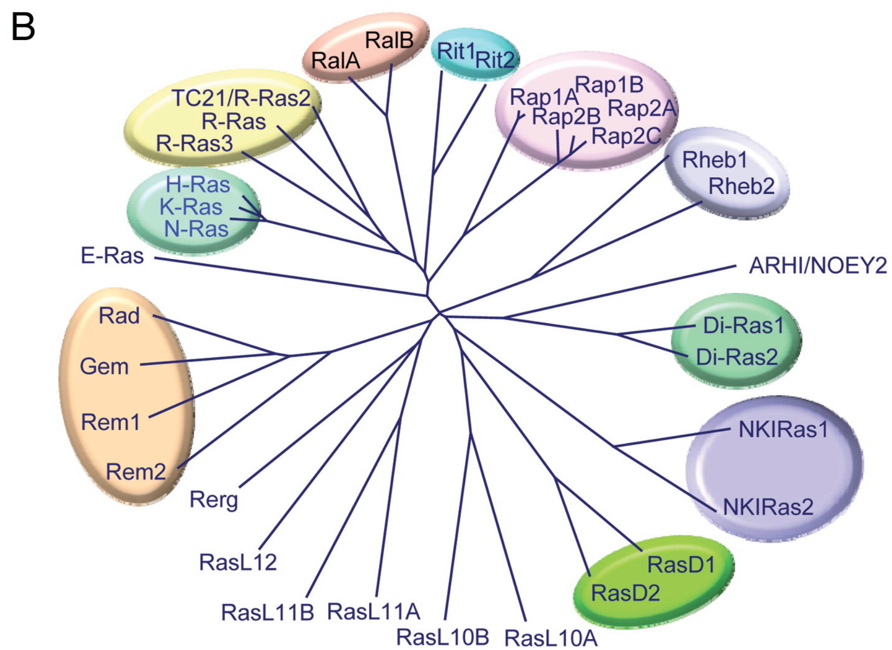
# Ras Superfamily

# In Progress – Determining the Role of the Superfamily of Ras-Related Small GTPases in Cancer

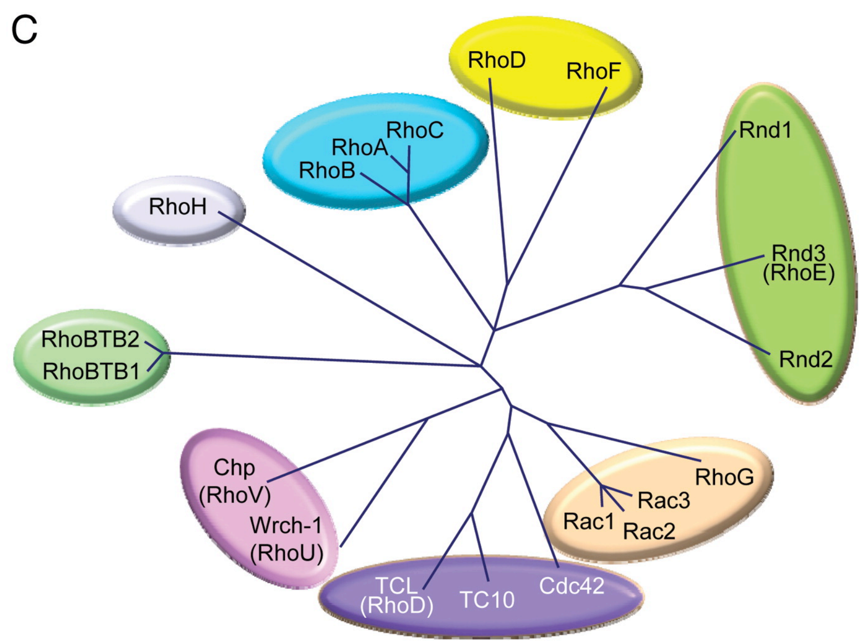



Cox AD and Der CJ. Ras history: The saga continues. Small GTPases 1:1, 2010

# In Progress: Role of Ras Superfamily Members in Cancer



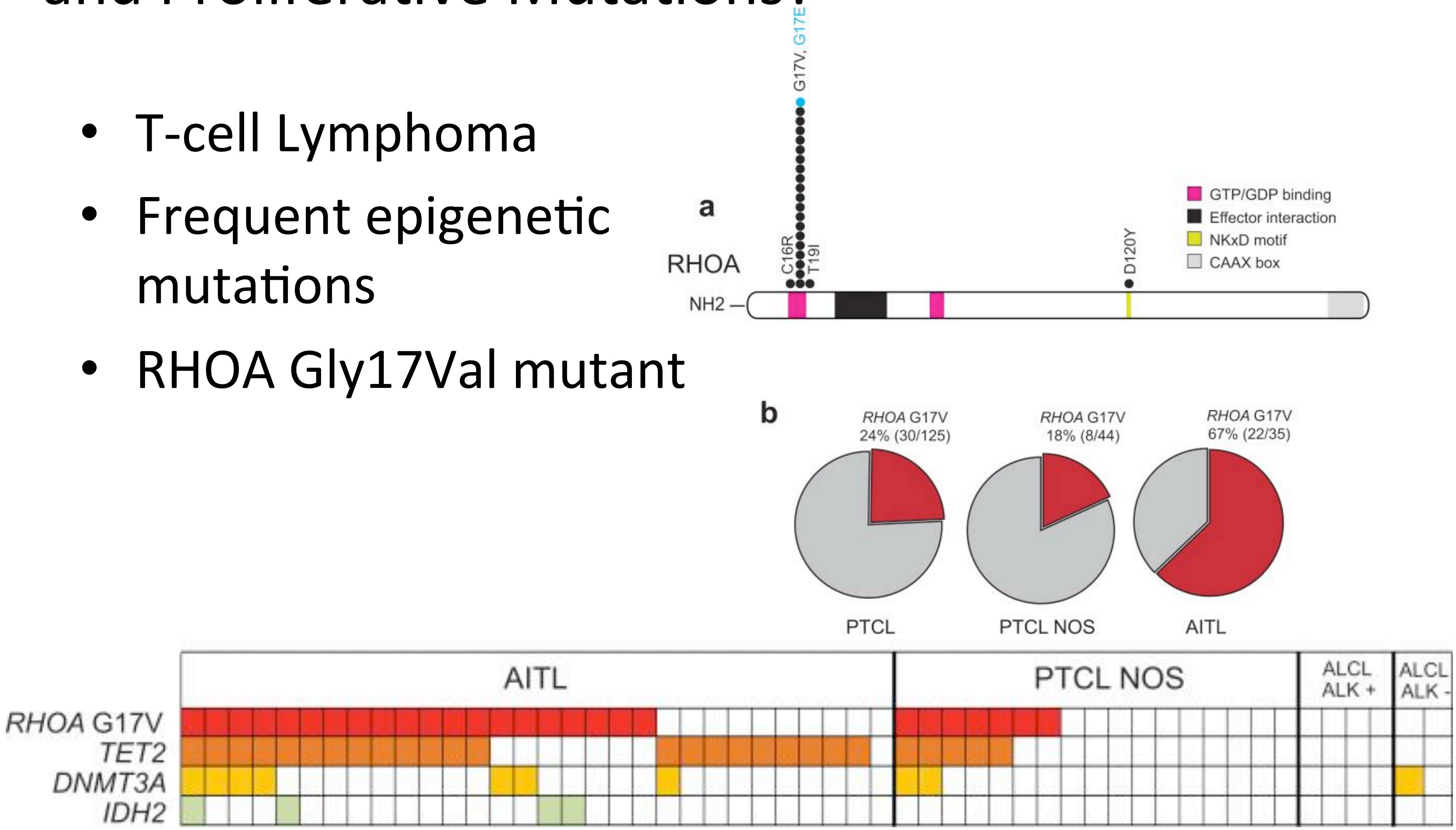

RAS Family




Rho Family

# Learning from the Ras AML Paradigm: Founder and Proliferative Mutations?

- T-cell Lymphoma
- Frequent epigenetic mutations
- RHOA Gly17Val mutant

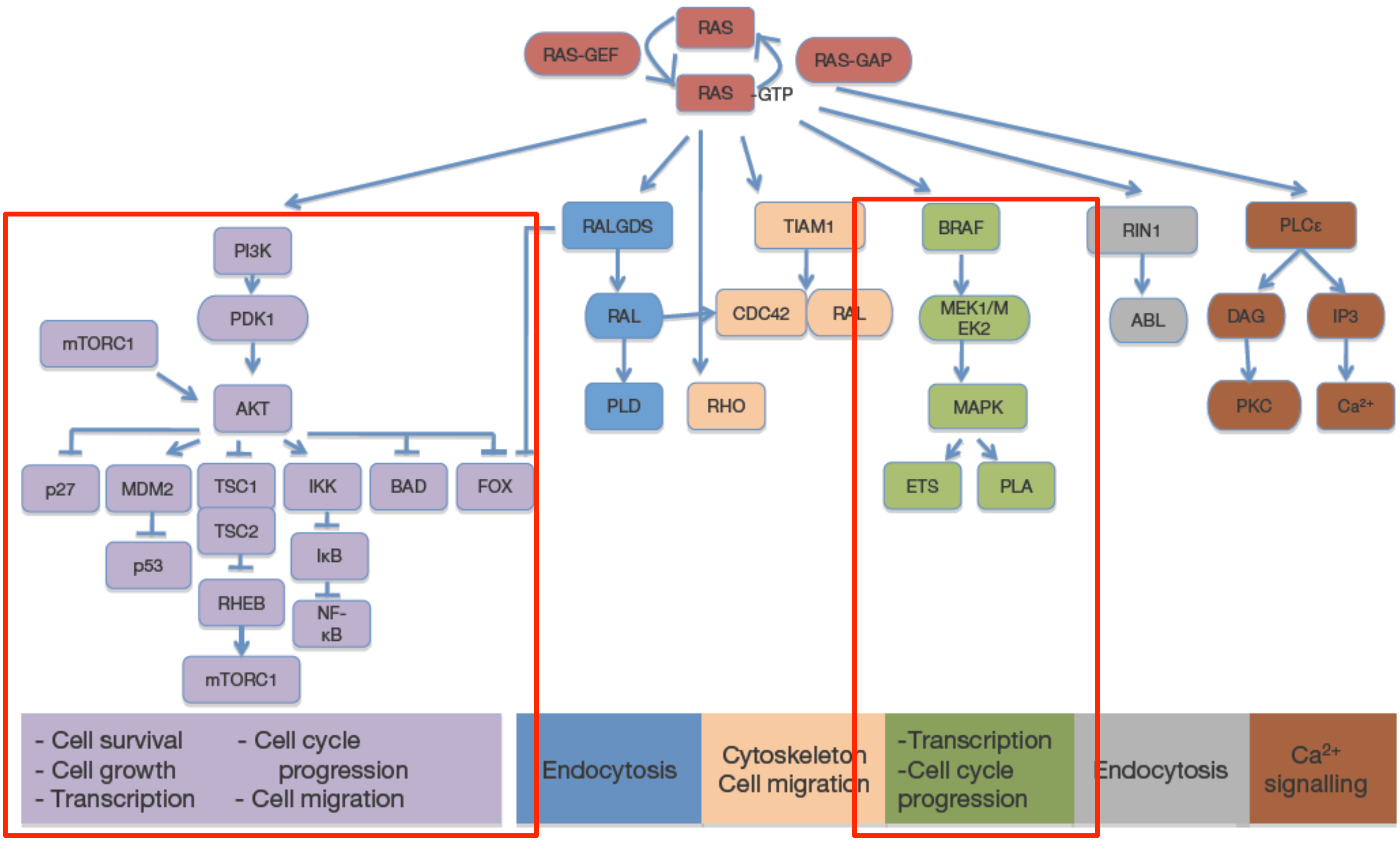



Palermo et al. Nature Genetics 46:166, 2014

# Ras as Therapeutic Target

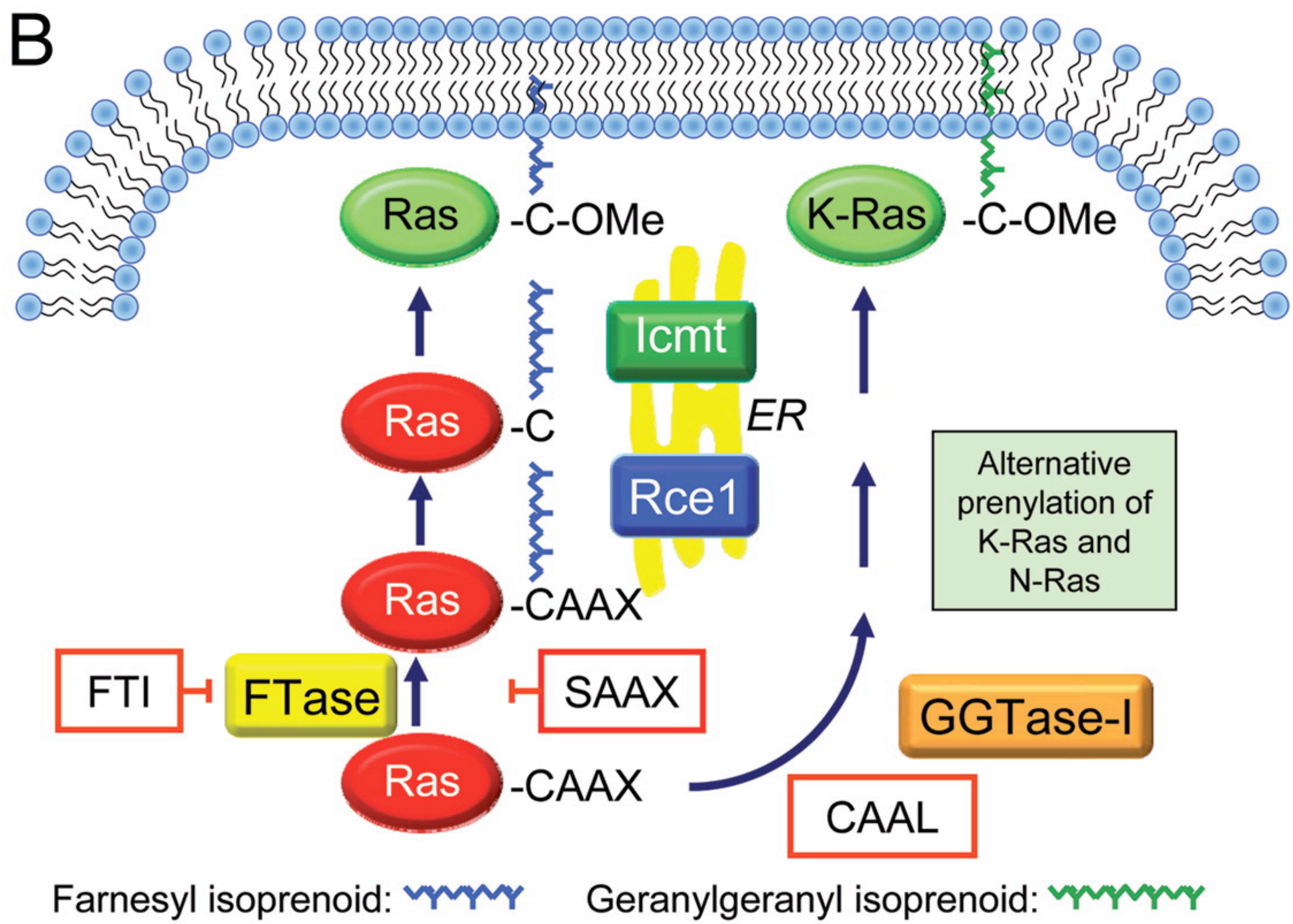
- MEK, AKT Inhibitors of Downstream Signaling
- RAS Farnesyl Transferase Inhibitors

# RAS mediates signaling through at least six different intracellular pathways

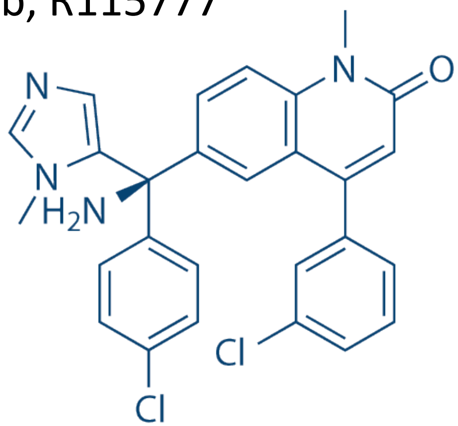



de Castro Carpeño J, et al. Transl Lung Cancer Res 2013;2(2):142-151

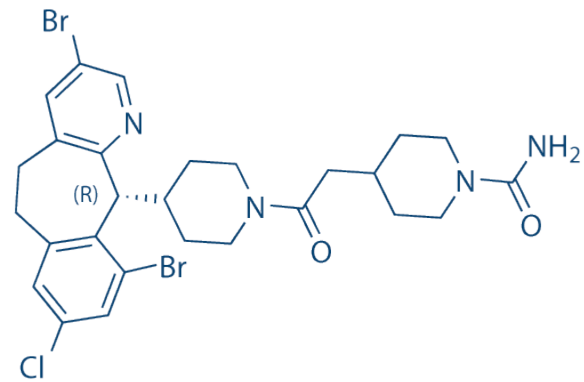
# Farnesylation and geranylgeranylation are post-translational modification required to recruit RAS to the cell membrane



Tipifarnib, R115777




Lonafarnib, SCH66336




Inhibits Ras farnesylation, Ras targeting to membrane, and cell proliferation
Early positive results in AML – Responses in 10 of 34 pts (w/o RAS mutations)
R115777 accumulated in bone marrow
Farnesylation of FT substrates lamin A and HDJ-2 confirmed

**Table 1. Farnesyltransferase inhibitors in clinical trials.**

| Drug | Company | Description | Developmental stage |
|---|---|---|---|
| Lonafarnib (SCH66336, Sarasar) | Schering-Plough | Synthetic tricyclic derivative of carboxamide, nonpeptidomimetic | Phase III |
| Tipifarnib (R115777, Zarnestra) | Johnson & Johnson | Imidazole-containing heterocyclic compound, nonpeptidomimetic | Phase III |
| L-778,123 | Merck | CAAX-competitive inhibitor, peptidomimetic | Phase I |
| BMS-214662 | Bristol-Myers Squibb | Tetrahydrobenzodiazepine, non-thiol, non-peptide small molecule inhibitor | Phase I |
| Salirasib | Concordia Pharmaceuticals | *S-trans,trans*-farnesylthiosalycilic acid, FTS, a synthetic small molecule | Phase II |

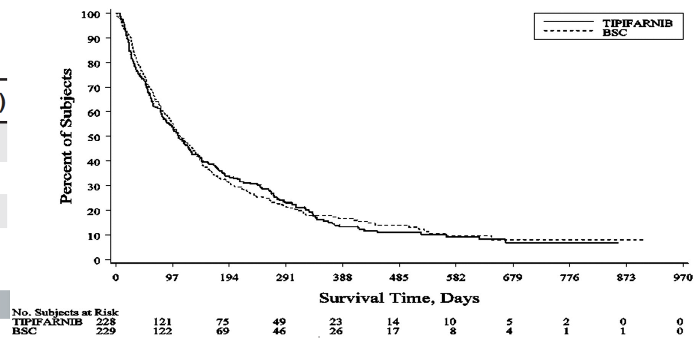
Karp JE et al., Blood 97:3361, 2001
Tsimberidou et al. Expert Opin Investig Drugs 19:1569, 2010

# FTI Development: Disappointment in AML

- AML
  - Tipifarnib vs BSC (best supportive care, incl. hydroxyurea) first-line elderly AML
    - Median OS 107 days vs 109 days.

**Table 3. Objective response rate for all randomized patients**

| Response | BSC (n = 229) | Tipifarnib (n = 228) |
|---|---|---|
| Complete response | 0 | 18 (8%) |
| Partial response | 1 (< 1%) | 6 (3%) |
| Hematologic improvement | 2 (1%) | 14 (6%) |
| Stable disease | 130 (57%) | 105 (46%) |
| Progressive | | |
| Not done/not | | |




NEWS CENTER

PAGE 6 / JUNE 10, 2005

## ODAC Votes Thumbs Down on Tipifarnib (Zarnestra) for Elderly Patients with AML

*By Margot J. Fromer*

ROCKVILLE, MD—The Oncologic Drugs Advisory Committee (ODAC) had strong reservations about not only the quality of the clinical trial presented in support of FDA approval of tipifarnib (Zarnestra) but also the very low response rate. The decision, then, was to refuse to recommend either full or accelerated approval.

*ODAC members were most concerned about the low response rate. "It's like Iressa all over again," said Otis Brawley, MD, who expressed the feelings of a number of other*

progress: a Phase II study to evaluate an alternate dosing and administration schedule, and a Phase III trial comparing tipifarnib with best supportive care.

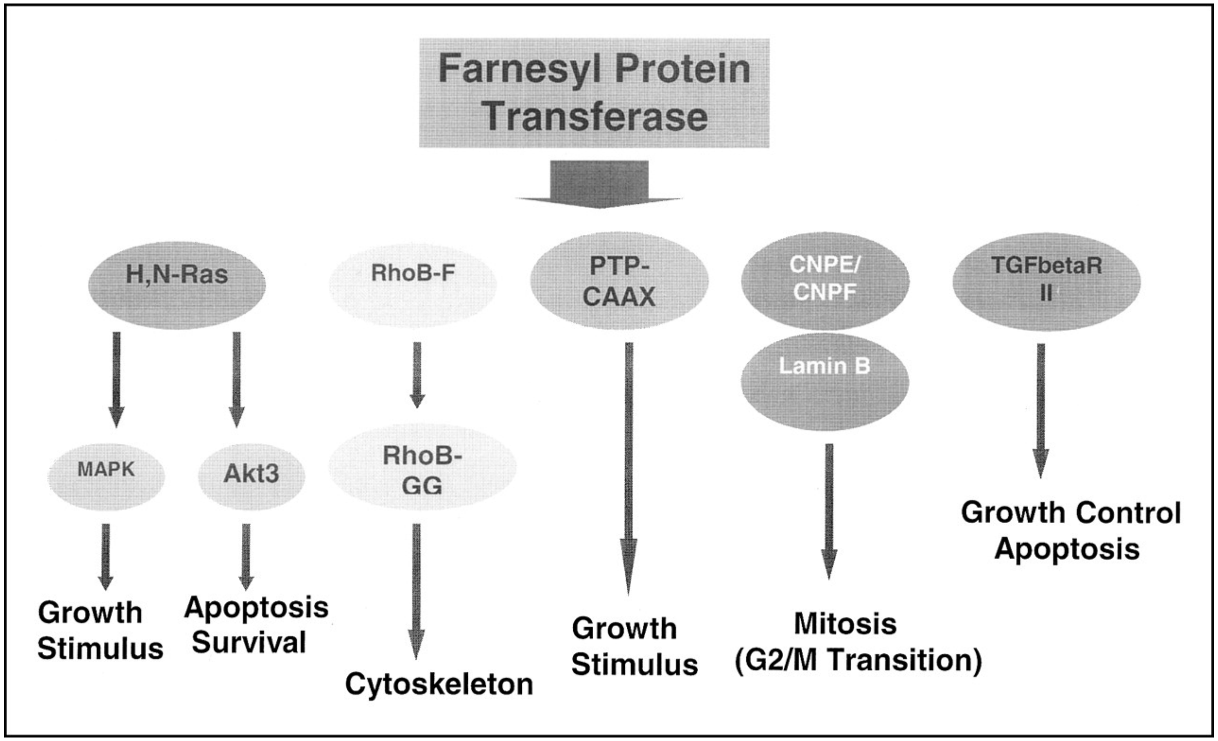
The one described at this meeting in support of approval was dubbed CTEP-20, a Phase II, open-label, single-arm study of 136 AML patients with a median age of 75. A total of 82% had prior myelodysplastic syndrome (MDS), 49% had unfavorable karyotypes, and 90% had two or more other

ity, and poor performance status.

"Their current options are limited," he continued: investigational studies, low-dose chemotherapy, or supportive care. This is a population with an unmet need, tipifarnib can be given on an outpatient basis, it has a positive ratio of benefit to risk, and it will provide a new treatment for people who are not good candidates for induction chemotherapy."

The Oncology Times, June 2005

# Multiple Farnesylated Intracellular Proteins May Contribute to the Antiproliferative Effects of Farnesyl Protein Transferase Inhibition




*Consistent with the observed ras-independent clinical activity of tipifarnib, alternative cellular targets of farnesyltransferase inhibition have been identified in preclinical experiments. Farnesylation inhibition of interesting candidate proteins might contribute to these observed antitumor properties; these proteins currently include RhoB, centromere-binding proteins E and F (CNP-E and CNP-F), lamin B, protein tyrosine phosphatase, and transforming growth factor beta receptor-II (Fig 1).*

Van Cutsem E et al. JCO 2004;22:1430-1438

# Zarnestra development halted: prediction signature failed

- Responses observed in absence of RAS mutation; downstream effectors down-regulated in AML. An alternate response signature was identified.
- NCT01361464: Complete Remission (CR) rate in Acute Myelogenous Leukemia (AML) patients prospectively selected for R115777R115777 (ZARNESTRA) treatment on the basis of a 2-gene signature (RASGRP1:APTX ratio) in bone marrow aspirates.
- CR Rate 11%
- The study opened to accrual on 5/24/2011 and closed to accrual 07/25/2012 when the pharmaceutical company decided to terminate further development of Tipifarnib in acute myeloid leukemia (AML).

EIGER
Leader in HDV

ABOUT EIGER PIPELINE ABOUT HEPATITIS D

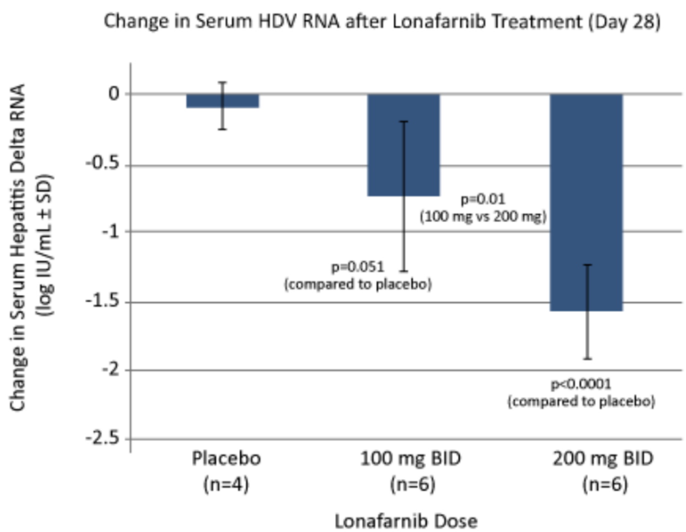
Press Releases

**EB Pharma Announces License Agreement for Investigational Drug, Tipifarnib from Janssen Pharmaceutica for Development in Hepatitis Delta Virus (HDV) Infection**

Palo Alto, December 23, 2014 /PRNewswire/ -- EB Pharma, LLC., a subsidiary of Eiger BioPharmaceuticals, Inc., today announced that it has executed an agreement with Janssen Pharmaceutica NV, ("Janssen"), for an exclusive license, to tipifarnib in the field of virology and a related, clinical stage back-up compound. EB Pharma is conducting clinical studies in patients infected with Hepatitis Delta (HDV) and will assess the efficacy and tolerability of tipifarnib as a potential new therapy.

Raponi et al. Blood 111: 2589, 2008
https://clinicaltrials.gov/ct2/show/NCT01361464

# Lonafarnib: New Life Inhibiting Hepatitis Delta Virus




**Description**

Lonafarnib is a well-characterized, late stage, orally active inhibitor of farnesyl transferase, an enzyme involved in modification of proteins through a process called prenylation. HDV uses this host cellular process inside liver cells to complete a key step in its life cycle. Lonafarnib inhibits the prenylation step of HDV replication and blocks the ability of the virus to multiply. Since prenylation is a host process, not under control of HDV, and lonafarnib inhibits prenylation, there is also a theoretical higher barrier to resistance with lonafarnib therapy. Virus mutation, a common pathway to drug resistance, is not expected to be a potential pathway to lonafarnib resistance by HDV.

# FTI Development: Disappointment in Pancreatic Cancer

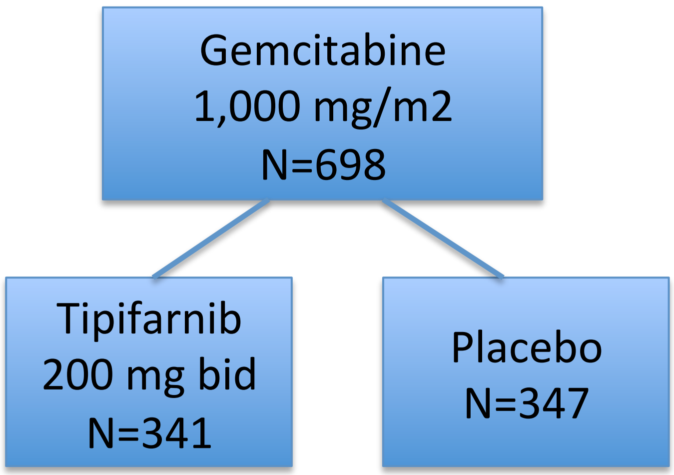



**Table 2.** Efficacy Parameters

| Efficacy | Tipifarnib + Gemcitabine (n = 341) | Placebo + Gemcitabine (n = 347) | *P* |
|---|---|---|---|
| Overall survival | | | |
| Median, days | 193 | 182 | .75 |
| 95% CI | 176 to 218 | 155 to 206 | |
| 6-month survival, % | 53 | 49 | |
| 1-year survival, % | 27 | 24 | |
| Progression-free survival | | | |
| Median, days | 112 | 109 | .72 |
| 95% CI | 105 to 119 | 101 to 118 | |
| Best response reconciled, % | | | |
| CR or PR | 6 | 8 | |
| Stable disease | 53 | 52 | |
| Progression | 28 | 30 | |
| Not assessable | 13 | 10 | |
| Time to PS deterioration, days | 142 | 125 | .50 |
| 95% CI | 121 to 176 | 107 to 144 | |

Abbreviations: CR, complete response; PR, partial response; PS, performance status.

Van Cutsem et al., J Clin Oncol 22:1430, 2004

# What do these observations teach us about KRAS and its therapeutic potential?

- It is not clear that KRAS mutation confers a worse outcome
- KRAS signaling interferes with EGFR signaling blockade

- KRAS role in oncogenesis remarkably is still being worked out
- Early data: RAS was not able alone to transform primary cells
- KRAS appears to  be the first event in pancreatic cancer
- KRAS mutant and non-mutant subclones often coexist
- KRAS mutation occurs in setting of “landscape mutation” in AML

- *KRAS is an important and critical target for cancer therapy, but its inhibition may not be sufficient in many cancer types*
- *We need definitive KRAS blockade to answer many of our questions about the role of KRAS in the origin and maintenance of cancer*

# Acknowledgements

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